Dissertation on

"A HOSPITAL-BASED CASE-CONTROL STUDY ON IRON DEFICIENCY AND ANAEMIA IN CHRONIC HEART FAILURE WITH REDUCED EJECTION FRACTION"

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CERTIFICATE

This is to certify that the dissertation titled **"A HOSPITAL-BASED CASE-CONTROL STUDY ON IRON DEFICIENCY AND ANAEMIA IN CHRONIC HEART FAILURE WITH REDUCED EJECTION FRACTION"** is the bonafide original work done by **Dr. TANUJ MOSES LAMECH**, post graduate student, Institute of Internal medicine, Madras medical college, Chennai-3, in partial fulfillment of the University Rules and Regulations for the award of MD Branch -1 General Medicine, under our guidance and supervision, during the academic year 2015-2018.

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DECLARATION

I, **Dr. TANUJ MOSES LAMECH** solemnly declare that dissertation titled **"A HOSPITAL-BASED CASE-CONTROL STUDY ON IRON DEFICIENCY AND ANAEMIA IN CHRONIC HEART FAILURE WITH REDUCED EJECTION FRACTION"** is a bonafide work done by me at Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-3 during March 2017 to August 2017 under the guidance and supervision of my unit chief **Prof. Dr. R.PENCHALAIAH M.D**, Professor of Medicine, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai. This dissertation is submitted to Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of **M.D. DEGREE IN GENERAL MEDICINE BRANCH-I**.

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ABBREVIATIONS

BNP	-	B-type natriuretic peptide
CONFIRM-HF	-	Ferric CarboxymaltOse evaluatioN on perFormance in patients with IRon deficiency in coMbination with chronic Heart Failure
ESC	-	European Society of Cardiology
FAIR-HF	-	Ferinject Assessment in Patients with Iron Deficiency and Chronic Heart Failure
FCM	-	Ferric carboxymaltose
HFmrEF	-	Heart failure with mid-range ejection fraction
HFpEF	-	Heart failure with preserved ejection fraction
HFrEF	-	Heart failure with reduced ejection fraction
HRQoL	-	Health related quality of life
ID	-	Iron deficiency
LVEF	-	Left ventricular ejection fraction
NYHA	-	New York Heart Association
sTfR	-	Soluble transferrin receptor

CONTENTS

Introduction

INTRODUCTION

"The very essence of cardiovascular practice is the early detection of heart failure." - Sir Thomas Lewis, 1933

Heart failure is a complex clinical syndrome that occurs as a result of functional or structural impairment of ventricular filling or ejection, and this in turn leads to classical symptoms and signs of heart failure. It is the ultimate consequence of a multitude of pathologies that affect left ventricular structure or function.

Over 20 million people worldwide are afflicted with heart failure, and the risk increases with age. The overall prevalence appears to be increasing, particularly because of improved survival from other cardiac ailments such as myocardial infarction and arrhythmias.

It has been observed that patients with heart failure frequently have associated iron deficiency, which may or may not be associated with anaemia. Furthermore, it has been established that this iron deficiency, irrespective of the presence of concomitant anaemia, is independently associated with exercise intolerance, poorer quality of life, and increased mortality in patients with heart failure.

Trials have been conducted to ascertain whether repletion of iron to deficient patients with heart failure improves outcomes, and two major trials, FAIR-HF and CONFIRM-HF have shown promising results.

The purpose of this study is to estimate the burden of iron deficiency and anaemia in patients who have heart failure with a reduced ejection fraction.

Aims and Objectives

AIMS AND OBJECTIVES

1. To determine the prevalence of iron deficiency in the study subjects with heart failure with reduced ejection fraction.

2. To determine the prevalence of anaemia in the study subjects with heart failure with reduced ejection fraction.

3. To ascertain whether there is a statistical association between iron deficiency and heart failure with reduced ejection fraction.

Review of Literature

REVIEW OF LITERATURE

History of heart failure:

Ancient texts from Egypt, Greece and India provide some of the earliest descriptions of heart failure. The foxglove was used for medicinal purposes by the Romans. Blood-letting and leeches as treatments, not just for heart failure, but also for a myriad of other conditions, were in use for centuries. However it was only after William Harvey first described the nature of circulation in 1628 that a scientific understanding of the condition was achieved.[1]

William Withering, in 1785, published his classic book, *An Account of the Foxglove and some of its Medical Uses*, which discussed 158 patients whom Withering had treated with the Foxglove. The active ingredient of the Foxglove is today known as digitalis, after the plant's Latin name, *Digitalis purpurea*.[2]

Southey's tubes, inserted into oedematous peripheries, were used in the 19th and early 20th centuries, to allow drainage of the interstitial fluid.

[Figure 1: Digitalis purpurea – the purple foxglove][1]

Definition of heart failure:

The 2016 European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic heart failure define heart failure as

"A clinical syndrome characterised by typical symptoms (e.g. breathlessness, ankle swelling and fatigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral oedema) caused by structural and/or functional cardiac abnormality, resulting in reduced cardiac output and/or elevated intracardiac pressures at rest or during stress"[3]

Thus the present definition is restricted to only clinically apparent heart failure. Prior to this symptomatic phase, there may be asymptomatic structural or functional abnormalities in the heart (such as left ventricular dysfunction which may be systolic or diastolic). These are considered precursors of heart failure. However, even these precursors are associated with poor outcomes, and initiation of pharmacotherapy may reduce mortality.[4, 5]

Heart failure with preserved, mid-range and reduced ejection fraction:

Heart failure occurring in patients with a normal left ventricular ejection fraction (typically ≥50%) is termed “heart failure with preserved ejection fraction” or HFpEF. This was previously called “diastolic heart failure”.

Similarly, heart failure occurring in patients with a reduced left ventricular ejection fraction (typically <40%) is termed “heart failure with reduced ejection fraction” or HFrEF. This was previously called “systolic heart failure”.

Patients with ejection fractions of 40-49% represent a grey area, which is now defined as “heart failure with mid-range ejection fraction” or HFmrEF.

HFrEF was previously referred to as “systolic heart failure” and HFpEF was previously referred to as “diastolic heart failure”. However, it is now acknowledged that most patients with HFrEF also have diastolic dysfunction, and patients with HFpEF may have subtle abnormalities in systolic function. It is for these reasons that the terms “systolic” and “diastolic” heart failure have fallen out of favour.

A majority of the trials and published clinical data since 1990 chose patients based on ejection fraction, and it is only in patients with HFrEF that therapies have been shown to reduce mortality and morbidity.[3]

Terminology related to time course of heart failure:

"Chronic heart failure" refers to patients who have had heart failure for some time.

"Stable" heart failure is used to describe treated patients with symptoms and signs that, for at least 1 month, have generally remained unchanged.

"Decompensated" heart failure describes patients with previously chronic stable heart failure who deteriorate, and this deterioration may happen suddenly or slowly.

Terminology related to symptomatic severity of heart failure:

Severity of symptoms is frequently graded by the New York Heart Association functional classification.

Class I	No limitation of physical activity. Ordinary physical activity does not cause undue breathlessness, fatigue, or palpitations.
Class II	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in undue breathlessness, fatigue, or palpitations.
Class III	Marked limitation of physical activity. Comfortable at rest, but less than ordinary physical activity results in undue breathlessness, fatigue, or palpitations.
Class IV	Unable to carry on any physical activity without discomfort. Symptoms at rest can be present. If any physical activity is undertaken, discomfort is increased.

[Fig 2: New York Heart Association functional classification of heart failure based on severity of symptoms and physical activity][3]

However, it must be borne in mind that the severity of symptoms correlates poorly with left ventricular function. Although patients with severe symptoms have decreased survival, it is also true that patients with mild symptoms also have an increased risk of hospitalisation and death.[6, 7, 8] The American College of Cardiology Foundation/American Heart Association (ACCF/AHA) classification is based on both structural changes as well as symptoms.[9]

A	At high risk for HF but without structural heart disease or symptoms of HF.
B	Structural heart disease but without signs or symptoms of HF.
C	Structural heart disease with prior or current symptoms of HF.
D	Refractory HF requiring specialized interventions.

[Fig 3: The American College of Cardiology Foundation/American Heart Association (ACCF/AHA) stages of heart failure][3]

Sometimes the phrase "advanced heart failure" is used to describe patients who have severe symptoms, severe cardiac dysfunction and recurrent decompensation.[10]

In the setting of an acute myocardial infarction, the clinical severity of the event may be graded by the Killip classification.[11]

Epidemiology of heart failure:

There is variability in the prevalence of heart failure depending upon the definition that is used. In developed countries, the prevalence is about 1-2% of the adult population, and this figure rises to >10% in people aged >70 years. The lifetime risk of developing heart failure by the age of 55 is approximately 28% for women and 33% for men.[12, 13, 14, 15] The present mortality rate after initial hospitalisation for heart failure is about 25-35% at 1 year.[22]

Diagnosis of heart failure:

Heart failure produces symptoms that are non-specific and these alone cannot differentiate heart failure from other diseases. Signs, such as displacement of the apical impulse, and a raised jugular venous pressure, are more difficult to detect, but may be more specific.[16, 17, 18] Interpretation of these signs and symptoms become difficult in elderly patients, obese individuals, and in patients with coexistent chronic lung disease.[19, 20, 21]

Type of HF		HFrEF	HFmrEF	HFpEF
CRITERIA	1	Symptoms ± Signs	Symptoms ± Signs	Symptoms ± Signs
	2	LVEF <40%	LVEF 40–49%	LVEF ≥50%
	3	–	1. Elevated levels of natriuretic peptides ; 2. At least one additional criterion: a. relevant structural heart disease (LVH and/or LAE), b. diastolic dysfunction	1. Elevated levels of natriuretic peptides ; 2. At least one additional criterion: a. relevant structural heart disease (LVH and/or LAE), b. diastolic dysfunction

[Fig 4: Definitions for heart failure with preserved (HFpEF), mid-range (HFmrEF) and reduced ejection fraction (HFrEF)

LVEF = left ventricular ejection fraction; LVH = left ventricular hypertrophy; LAE = left atrial enlargement][3]

Symptoms	Signs
Typical	**More specific**
Breathlessness Orthopnoea Paroxysmal nocturnal dyspnoea Reduced exercise tolerance Fatigue, tiredness, increased time to recover after exercise Ankle swelling	Elevated jugular venous pressure Hepatojugular reflux Third heart sound (gallop rhythm) Laterally displaced apical impulse
Less typical	**Less specific**
Nocturnal cough Wheezing Bloated feeling Loss of appetite Confusion (especially in the elderly) Depression Palpitations Dizziness Syncope Bendopnea[53]	Weight gain (>2 kg/week) Weight loss (in advanced HF) Tissue wasting (cachexia) Cardiac murmur Peripheral oedema (ankle, sacral, scrotal) Pulmonary crepitations Reduced air entry and dullness to percussion at lung bases (pleural effusion) Tachycardia Irregular pulse Tachypnoea Cheyne Stokes respiration Hepatomegaly Ascites Cold extremities Oliguria Narrow pulse pressure

[Fig 5: Symptoms and signs typical of heart failure][3]

Iron deficiency and heart failure:

Iron deficiency is common in patients with HFrEF, and is associated with a poorer quality of life and increased mortality in these patients. This is true of iron deficiency even in the absence of concomitant anaemia.[23, 24] 751 patients with stable chronic heart failure were studied in a multi-ethnic Asian populations, and iron deficiency was found in 61.4% of them, as against a 39.3% prevalence in controls from the general population.[25] Another study involving 127 patients with stable chronic heart failure with a left ventricular ejection fraction of <45%, about one-third of study participants were found to be iron deficient. Of these iron deficient individuals, 75% were not anaemic.[26]

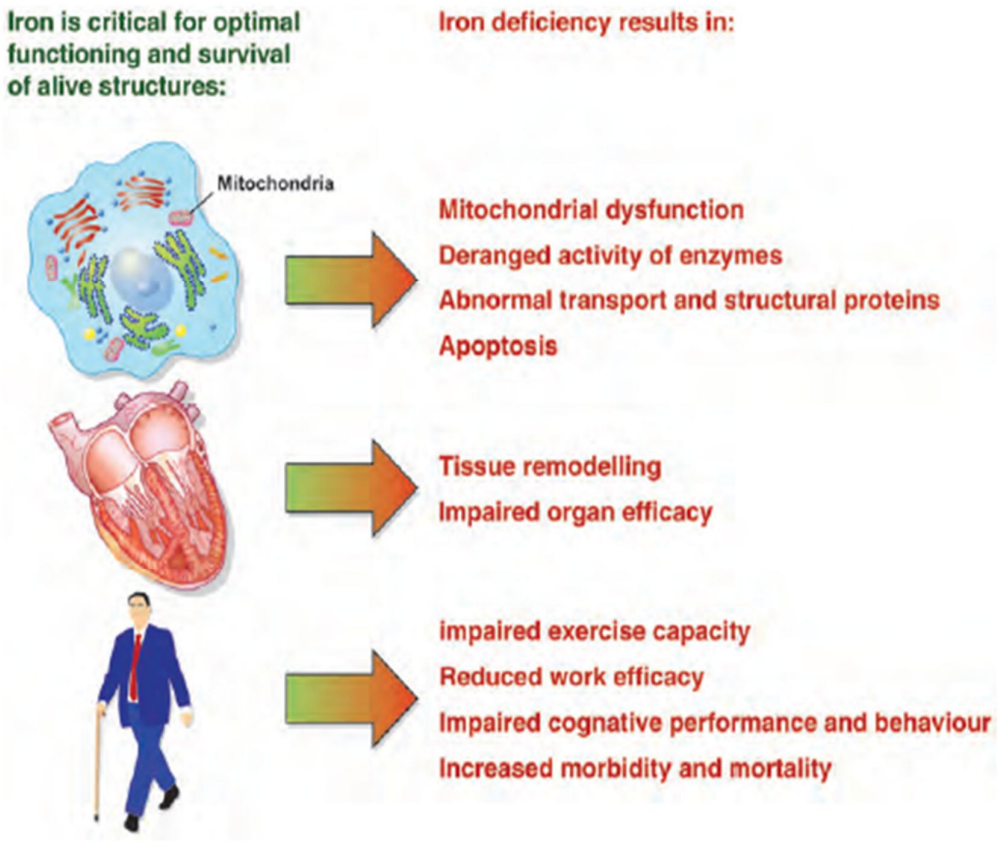
Study	*n*	LVEF cohort average (%)	Age range (years)	Ethnicity	Prevalence of ID		
					Whole group (%)	Non-anaemic patients (%)	Anaemic patients (%)
Yeo *et al.*	751	34.4 ± 15.9	62 ± 12.2	Asian	61.4	59	65.3
Rangel *et al.*	127	28 ± 9.1	53–68	Portugese	36	34	43
Jankowska *et al.*	546	26 ± 7	55 ± 11	European	37	32	57
Klip *et al.*	1506	33 ± 14	64 ± 13	European	50	45.6	61.2
Okonko *et al.*	157	32 ± 9	71 ± 12	British	69	65	78
Studies with ID defined by BM examination							
Nanas *et al.*	37	22.5 ± 5.9	57.9 ± 10.9	European	73	N/A	N/A

[Fig 6: Studies reporting prevalence of iron deficiency in heart failure][31]

Metabolism of iron:

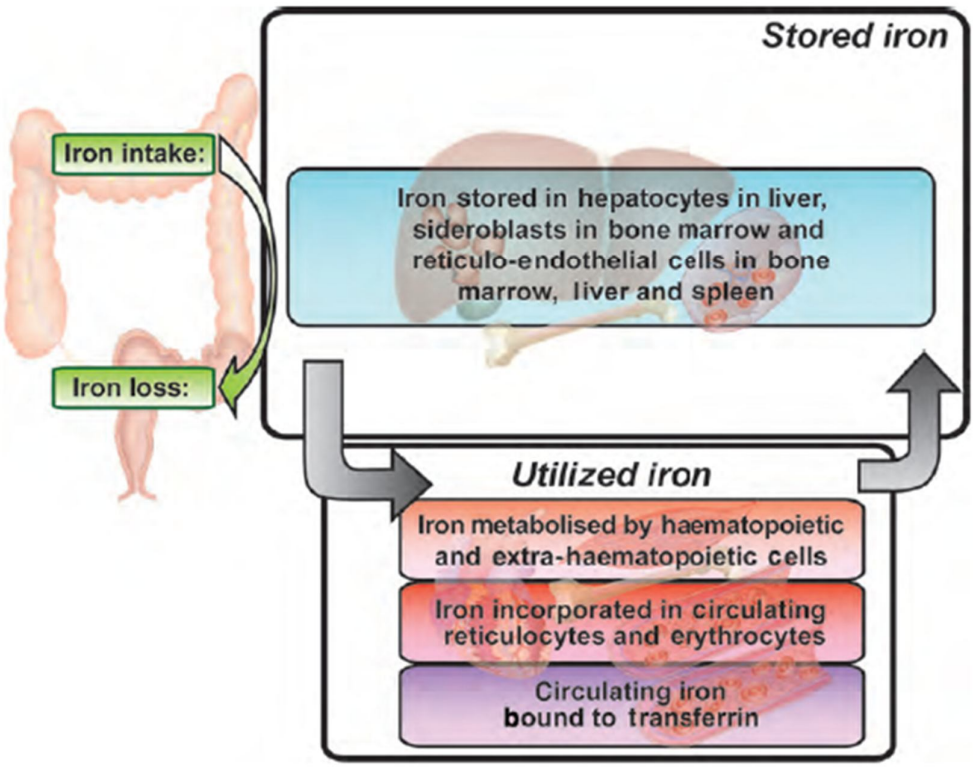
Iron is an active micronutrient. It is one of most important biological catalysts in the body.[27] It is an integral part of haemoglobin (Hb) and myoglobin, acts as a co-factor in the biochemical pathways of oxidative metabolism, and is involved in host defence mechanisms.[28, 29]

Except for sloughing of duodenal enterocytes and bleeding, there is no natural mechanism for active excretion of iron. Therefore, regulation of iron balance occurs during intake, recycling and storage. Haem-specific receptor (HCP1), located in the duodenal enterocyte, absorbs organic haeme, which is then broken down intracellularly to yield biliverdin and free iron.[30] The divalent metal transporter 1 (DMT1) absorbs non-haeme inorganic iron, which exists in the trivalent ferric (Fe^{3+}) form. This is then reduced by ferric oxidoreductases such as the duodenal cytochrome B, to the divalent ferrous (Fe^{2+}) form before absorption from the apical surface of the enterocyte. Intracellularly, some of these ferrous ions conjugate with apoferritin to form ferritin, which remains within the enterocyte. The rest are converted back into their trivalent ferric state and excreted into the bloodstream via ferroportin which is present in the basolateral surface of the enterocyte. This iron is then transported into the target tissues for storage and utilisation.[31]



[Fig 7: Importance of iron for functioning and survival across all levels of complexity of living structures][29]

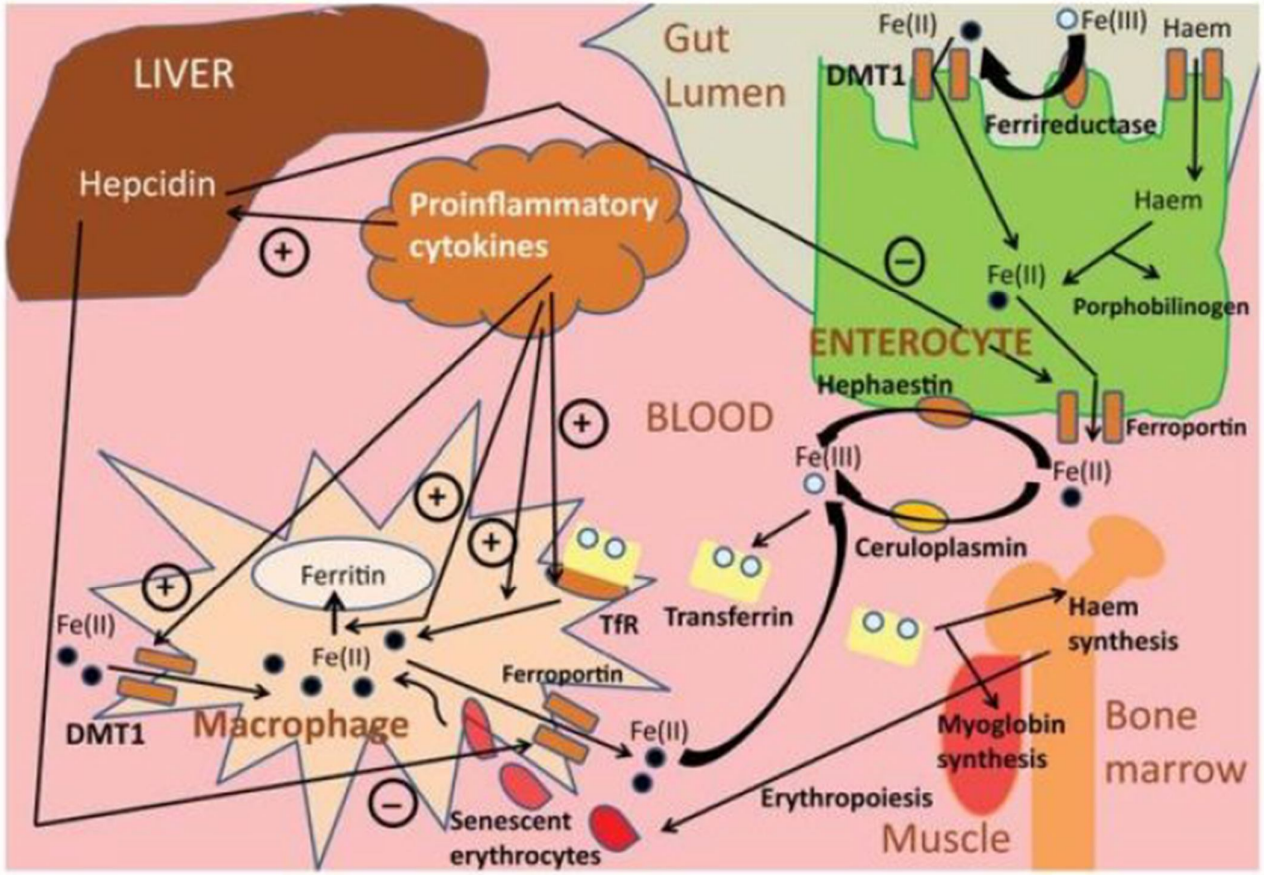
Ferroportin is a protein that exports iron from the cell, and is present in duodenal enterocytes, hepatocytes (which are the sites of iron storage) and in macrophages (which are the sites of iron recycling from senescent RBC's). The function of ferroportin is regulated by a liver-derived hormone called hepcidin, which is produced in response to elevated cytokine levels (especially IL-6) and fluctuating levels of iron within the hepatocyte.[32, 33]



[Fig 8: Major pools of utilised and stored iron in the body][29]

Hepcidin was postulated to deprive pathogens of essential iron during times of inflammation and infection, thus contributing to our innate immunity. Whenever cytokine or iron levels are elevated, hepcidin is synthesised, and this results in ferroportin being removed from the basolateral portions of the duodenal enterocytes, hepatocyte cell membranes, and macrophages, diminishing the efflux of iron into the systemic circulation.

In iron deficiency and hypoxia, however, there is down-regulation of hepcidin, and this permits ferroportin to remain incorporated in the cell membranes of hepcidin-sensitive cells, allowing increasing amounts of iron to be absorbed and released into the bloodstream. There appears to be some unidentified iron sensing mechanism which is yet to be elucidated, that somehow activates production and release of hepcidin.



[Fig 9: Possible mechanisms of iron deficiency in heart failure.

Fe(II), iron in ferrous form; Fe(III), iron in ferric form; DMT1, divalent metal transporter 1; TfR, transferrin receptor][55]

Defining iron deficiency:

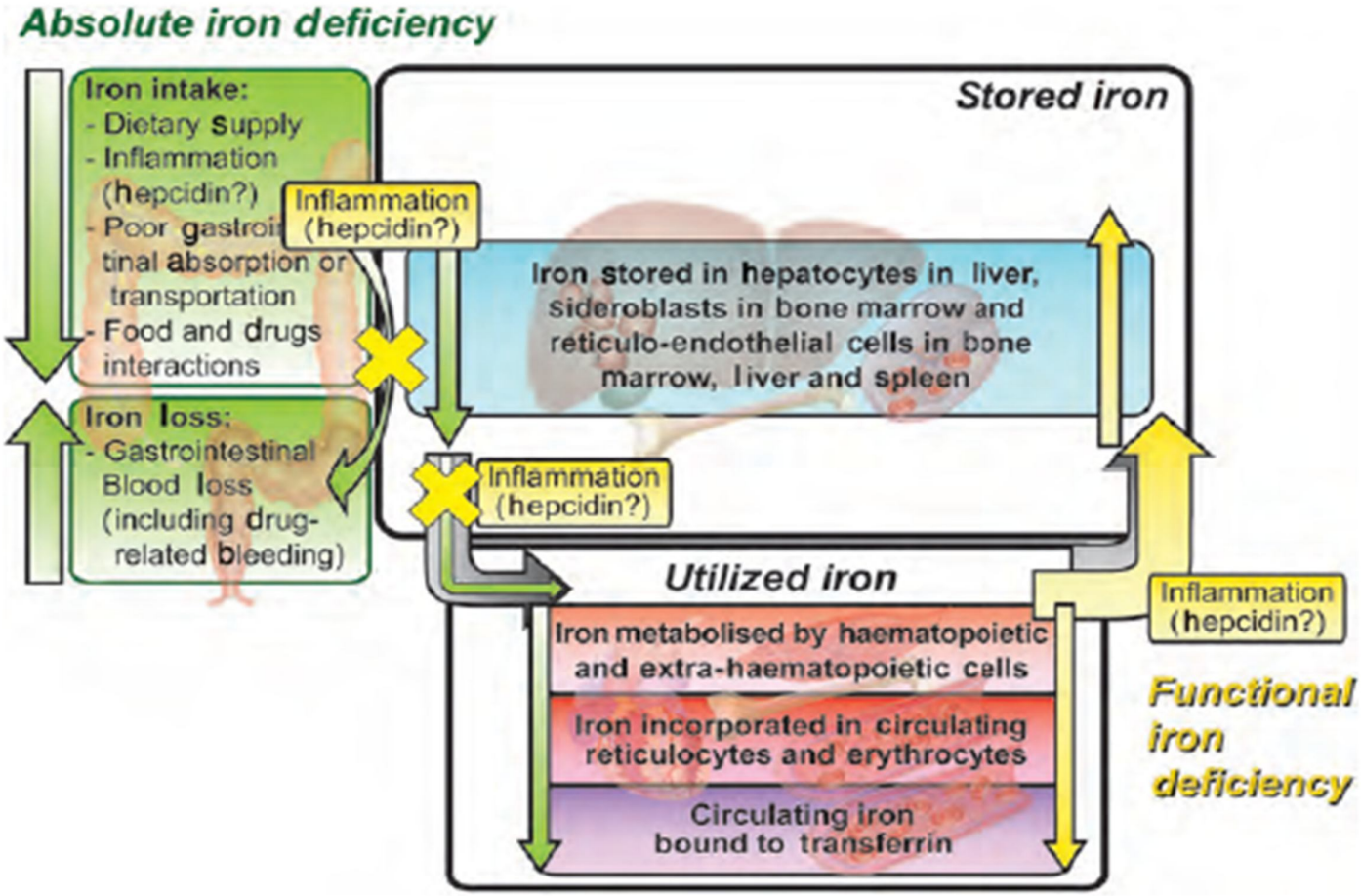
Iron deficiency (ID) is broadly classified as functional iron deficiency and absolute iron deficiency. It is only when this iron deficiency becomes severe enough to decrease erythropoiesis and reduce haemoglobin production that anaemia occurs.

In absolute iron deficiency (absolute ID), iron homeostasis is intact, but the iron stores are depleted. The gold standard for diagnosis remains bone marrow examination revealing an absence of iron with the use of specific stains such as Perls Prussian blue.[34] It was found that serum ferritin levels were an accurate reflection of iron stores in the bone marrow in the well patient. Iron deficiency can therefore be diagnosed if circulating ferritin level is lower than the reference range for the assay that is used. This is usually less than 15-30 µg/L.[35] However, since ferritin is an acute phase reactant, it has been found that in some patients with stable but chronic inflammatory conditions, absent iron stores in the bone marrow occur despite a serum ferritin of 70-98 µg/L.[36]

Functional iron deficiency (functional ID) occurs when, despite potentially normal body iron stores, there is still an inadequate iron supply to meet the demand. The most accurate measure of this functional iron deficiency is a transferrin saturation <20%. This is because transferrin saturation represents the immediately available circulating iron that can be

used for metabolism. Unlike ferritin which is a positive acute phase reactant, transferrin is a negative acute phase reactant. However, it is not affected by inflammation to the same degree as ferritin is.[37]

The serum level of soluble transferrin receptor (sTfR) has been suggested as being a more accurate measure of a person's iron status, because it is not affected by inflammation.[23, 39] Whenever intracellular stores of iron are diminished, there is an increase in production of transferrin receptor, so as to aid cellular uptake of iron. A recent meta-analysis found that, when compared with bone marrow iron staining, the diagnostic accuracy of serum soluble transferrin receptor (sTfR) for iron deficiency appears to have a lower specificity than ferritin, but a higher sensitivity.[40] However, it is difficult to make definitive conclusions about the relative accuracies of ferritin and soluble transferrin receptor (sTfR), as existing studies are heterogeneous, and the diagnostic cut-off for ferritin was not always adjusted to take into account the presence of inflammation.



[Fig 10: The concept of absolute and functional iron deficiency][29]

There does not exist a single perfect test to diagnose iron deficiency.

- Regular bone marrow biopsies are not practical
- Iron studies are easily available, but there is poor sensitivity in the presence of associated inflammation
- Soluble transferrin receptor (sTfR) is not widely available, and might lack specificity
- Other markers such as mean corpuscular volume [MCV] and red cell distribution width [RDW], have not been validated for use in the diagnosis of iron deficiency

Pragmatically, therefore, several recent clinical studies[25, 26, 41, 46, 50, 53, 54, 57] define iron deficiency as

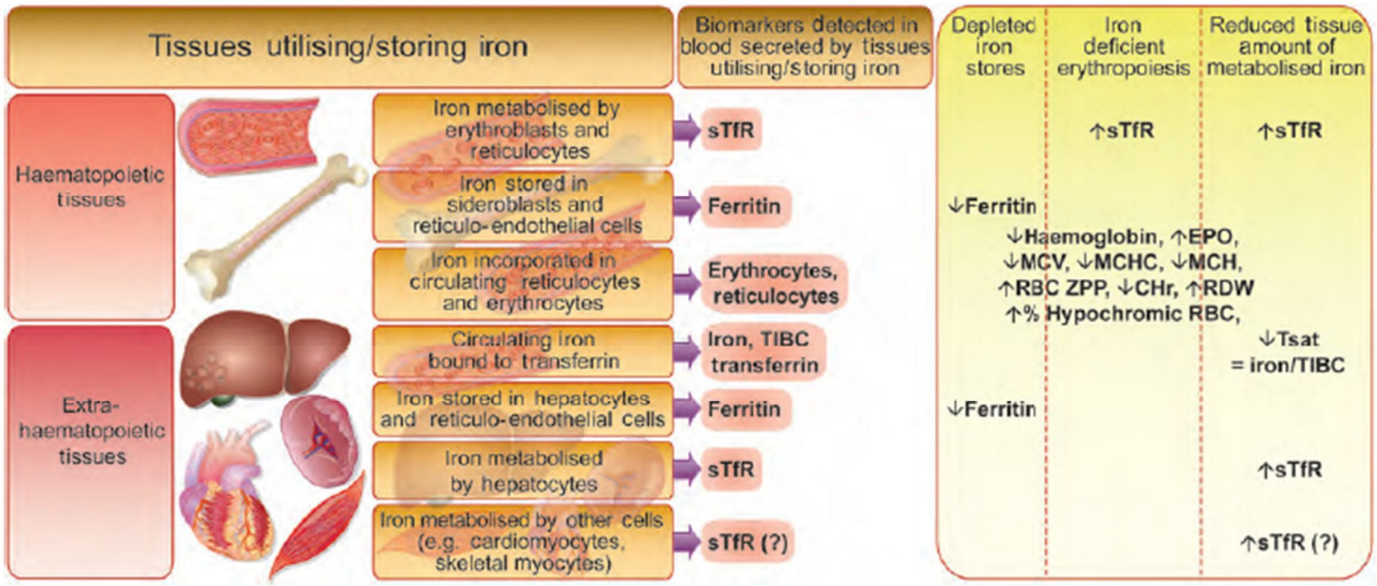
- Ferritin <100 µg/L (OR)
- Normal ferritin (100-300 µg/L) with a transferrin saturation <20%

	Absolute ID	Functional ID
Peripheral blood tests		
Ferritin	<100 µg/L	100–300 µg/L
Transferrin saturation	Tsat decreased but the level does not contribute to diagnosis	Tsat <20%
BM examination		
Prussian Blue-stained BM aspirates for the presence or absence of iron granules has been considered the gold standard in evaluating iron-depleted states		

[Fig. 11: Diagnosing iron deficiency][31]

It has been found that the earlier stages of heart failure are associated with functional iron deficiency, while the later more advanced stages of heart failure are associated with absolute iron deficiency.[27]

It is presently not known if sex-specific definitions of iron deficiency are needed.



[Fig. 12: Tissues utilising and/or storing iron and related biomarkers which are secreted by these tissues and can be detected in peripheral blood][29]

Pathophysiology of iron deficiency in heart failure:

Anaemia of chronic disease occurs due to inappropriate elevation of hepcidin. This rise in hepcidin correlates with high levels of inflammatory markers (such as IL-6) and greater disease severity.[44] However, in heart failure, the opposite is true, with hepcidin levels being inversely proportional to the degree of disease severity. In a case-control study that involved 321 patients with chronic heart failure, patients with mild symptoms (NYHA Class I/II) were found to have high hepcidin levels.[24] Ferritin levels were high and IL-6 levels were low, indicating that the high hepcidin was due to high iron levels, implying deranged iron homeostasis (functional iron deficiency). This was generally not associated with anaemia. However, patients with more severe disease had more iron-deficiency anaemia, and their hepcidin levels were low despite having associated elevations in IL-6. This suggests that hepcidin is more responsive to iron than to the inflammatory markers such as IL-6 in patients with heart failure. These findings were replicated by a similar study but with a smaller cohort.[45]

Liver congestion may also provide an explanation for the development of absolute iron deficiency in heart failure patients. An animal study induced iron deficiency anaemia in rats by either haemolysis, blood loss or liver congestion. It was found that the rats with liver congestion had lower transferrin and serum

iron levels, with higher hepcidin, compared to the other groups, regardless of the severity of anaemia.[33] The authors of the study postulated that liver congestion resulted in localised inflammation, which resulted in a high intra-hepatocyte iron content, and this stimulated inappropriate hepcidin release. This resulted in sequestering of systemic iron, and decreased gastrointestinal absorption, which eventually result in iron deficiency anaemia. Whether these findings can be extrapolated to humans with heart failure remains to be determined.

Myocardial iron homeostasis:

Cardiac myocytes have high energy requirements, and they are therefore highly susceptible to iron deficiency and abnormal iron utilisation.[46] It has been demonstrated that patients with end-stage heart failure who have been referred for cardiac transplantation, have depleted myocardial iron stores, as compared with healthy hearts. Also, soluble transferrin receptor (sTfR) levels were lower in the failing hearts that were explanted during transplantation, as compared with the levels in donor hearts that were deemed unsuitable for transplantation.[39] Exposure to aldosterone and noradrenaline, neurohormones that are commonly elevated in heart failure, resulted in a further reduction in the expression of soluble transferrin receptors (sTfR).[47] Animal models have shown that sustained iron deficiency can result in left ventricular dilatation and

hypertrophy, sarcomere disruption, mitochondrial swelling, and release of reactive oxygen species which can result in cell injury.[48]

Thus, heart failure affects multiple points in the regulatory pathway of iron

- Gut interstitial oedema decreases absorption of iron
- Liver congestion and chronic inflammation affects hepcidin regulation, reducing availability of iron for metabolism
- Neurohormones such as aldosterone and noradrenaline result in reduced expression of transferrin receptor in the cardiac myocyte, causing reduced intracellular iron availability. This in turn decreases the functional ability of the myocyte and increases the risk of apoptosis. All of these further stress the already failing myocardium.

Further research is still required to draw definitive conclusions about the role of iron in the pathophysiology of heart failure.

Risk factors for iron deficiency in patients with heart failure:

The patients with heart failure that have the highest risk of concomitant iron deficiency are

- Women
- Non-Caucasian patients
- Older patients

- Anaemic patients
- Patients with more severe disease

It has also been shown that high-sensitive C-reactive protein and NTpro-BNP levels independently correlate with iron deficiency anaemia in patients with chronic heart failure.

Pharmacotherapy with anti-coagulants or anti-platelet agents has not been associated with iron deficiency. This finding suggests that occult gastrointestinal bleeding is unlikely to be a dominant cause of iron deficiency anaemia in these patients. Angiotensin-converting enzyme inhibitors are known to correlate with the degree of anaemia in patients with heart failure. However, a similar correlation with iron deficiency does not appear to exist.

Clinical relevance of iron deficiency in heart failure:

Iron deficiency and quality of life:

Compared with healthy patients and even those with other chronic illnesses, patients with heart failure have a lower 'Health-related quality of life' (HRQoL).[49] This is primarily because of the limitations in performing activities of daily living. A cross-sectional study that involved 1278 patients in Europe who had heart failure, revealed that iron deficiency had a negative impact on HRQoL, independent of the presence of coexisting anaemia.[50] Among the 1278 patients, 35% of the patients were anaemic, but 58% had iron deficiency. The

HRQoL was measured with the Minnesota Living with Heart Failure questionnaire, and was found to be worse in patients with iron deficiency (with or without concomitant anaemia), as compared to those with neither iron deficiency nor anaemia. An earlier-reported analysis also made similar conclusions.[51]

Iron deficiency and exercise capacity:

A cardinal symptom of heart failure is exercise intolerance, and this has been found to be associated with high mortality and morbidity, along with a poorer quality of life.[52] It has been demonstrated that exercise tolerance is reduced in patients with iron deficiency, both with and without heart failure.

Iron deficiency and mortality:

Irrespective of the presence of concomitant anaemia, iron deficiency seems to be an independent predictor of mortality in patients with heart failure.[46] In one study, patients with iron deficiency anaemia were found to be at a 4-fold higher risk of death than patients who were iron replete. Even patients who were non-anaemic, but who had iron deficiency, were found to be at a 2-fold higher risk of death, compared with anaemic non-iron-deficient patients. This suggests that iron deficiency, more than anaemia, is an ominous finding in a patient with chronic heart failure.

Evidence for treating iron deficiency:

It has already been established in the available literature that the presence of iron deficiency is a common finding in patients with heart failure, and this in turn correlates with an impaired exercise capacity, a poorer health related quality of life (HRQoL), and is an independent predictor of mortality. In view of these conclusions, the role of correcting iron deficiency in patients with heart failure has recently become an area of interest, with several trials having been conducted. All trials, however, have only used various formulations of intravenous iron. Hence the role of oral iron replacement has not been assessed in patients with heart failure, and no comment can be made regarding the therapeutic efficacy and safety of this approach.

In the FAIR-HF trial[53], it was found that intravenous ferric carboxymaltose (FCM) improved quality of life, NYHA class (over 6 months), and self-reported patient global assessment. This was true in both non-anaemic and anaemic patients with heart failure.

In the CONFIRM-HF trial[54], intravenous ferric carboxymaltose was found to improve exercise capacity over a period of 24 weeks. It also decreased the risk of hospitalisations for heart failure.

Study	n	Inclusion criteria	Age (years)	Treatment	FU	Endpoints and results
Bolger *et al.*	16	NYHA class II–III Systolic HF ID and anaemia Design: non-R, no control group	68.3 ± 11.5	Iron sucrose 200 mg on days 1, 3, and 5	92 days	Improved NYHA class [all II at study end ($P < 0.002$)] Improved MLWHF score (33 ± 19 to 19 ± 14, $P = 0.02$) Improved 6MWD (242 ± 78 to 286 ± 72, $P = 0.01$) Increased Hb, iron and ferritin levels
Gaber *et al.*	40	NYHA class II–III LVEF ≤ 40% ID and anaemia Design: non-R, no control	57 ± 13	Iron dextran 200 mg (i.v.) weekly (until ferritin 200–300 μg/L or Tsat 30–40%)	12 weeks	Myocardial function: E/E′ decreased (BL 22 ± 3, week 12 13 ± 3, $P < 0.001$) Peak systolic strain rate improved (BL −0.72 ± 0.11, week 12 −1.09 ± 0.37, $P < 0.01$) Functional capacity: Improvement in NYHA class (BL 3.0 ± 0.4, week 12 2.1 ± 0.3, $P < 0.05$)
Toblli *et al.*	40	NYHA class II–IV LVEF ≤ 35% ID and anaemia Design: R DB PC	74 ± 8 (control) 76 ± 7 (treated)	Iron sucrose 200 mg (iv) weekly for 5 weeks	6 months	Primary: Increase in Hb, ferritin, tsat and creatinine clearance ($P < 0.01$), reduced NT-proBNP ($P < 0.01$), CRP ($P < 0.01$) Secondary: Improved NYHA class, 6MWD ($P = 0.01$), QOL and fewer hospitalizations ($P = 0.01$)
Okonko *et al.*	35	NYHA class II–III LVEF ≤ 45% ID with or without anaemia Design: R, open control	62 ± 11 (control) 64 ± 14 (treated)	Iron sucrose 200 mg (iv) weekly for 16 weeks or until ferritin >500 ng/mL	18 weeks	Primary: Increase in absolute pVO_2 96 mL/min ($P = 0.08$) Secondary: Increase in pVO_2 by 2.2 mL/kg/min ($P = 0.01$), Improvement in NYHA functional class ($P = 0.007$), MLHFQ score ($P = 0.07$), fatigue score ($P = 0.004$)
Anker *et al.*	459	NYHA class II–III LVEF ≤ 40% ID with or without anaemia Design: R DB PC	67.8 ± 10.3 (treated) 67.4 ± 11.1 (placebo)	Ferric carboxymaltose 200 mg weekly until iron replaced then 200 mg 4 weekly	24 weeks	Primary: Patient Global Assessment improved (OR 2.51, 95% CI 1.75, 3.61, $P < 0.001$); NYHA class improved (OR 2.40, 95% CI 1.55, 3.71 $P < 0.001$) Secondary: Improved 6MWD ($P < 0.001$), KCCQ score ($P < 0.001$) and EQ-5D score ($P < 0.001$)
Ponikowski *et al.*	304	NYHA class II–III LVEF ≤ 45% ID with or without anaemia Design: R DB PC	68.8 ± 9.5	Ferric carboxymaltose 500–2000 mg at week 1 and 6 (+500 mg at weeks 12, 24, 36 if still ID)	52 weeks	Primary: Improved 6MWD at wk 24 (+33 ± 11 m, $P = 0.002$) Secondary: Improvement in NYHA functional class, PGA score, KCCQ score, 6MWD at weeks 36 and 52 (36 ± 11 m, $P < 0.001$), decreased risk of hospitalization for worsening HF (HR 0.39, 0.19–0.82, $P = 0.009$)

6MWD, 6-min walk distance; BL, baseline; ID, iron deficiency; KCCQ, Kansas City Cardiomyopahty Questionnaire; LVEF, left ventricular ejection fraction; MLWHF, Minnesota Living with Heart Failure questionnaire; NYHA, New York Heart Association function class; Study design; R, randomised; DB, double-blind; PC, placebo-controlled.

[Fig. 13: Studies assessing the efficacy of iron replacement in patients with heart failure and iron deficiency][31]

It must be emphasised, however, that the administration of IV iron differed in the FAIR-HF and CONFIRM-HF trials. In the FAIR-HF trial, patients received infusions of ferric carboxymaltose weekly. The total dose was calculated as per Ganzoni's formula and this was then provided over a period of 3-7 weeks, with a median of 6 injections. In the CONFIRM-HF trial, however, ferric carboxymaltose was given in large doses of 500-100 mg, based on haemoglobin and patient weight, as per a scheduled dosing scheme.

Haemoglobin (g/dL)	Weight (kg)	Dose of ferric carboxymaltose depending on visit		
		Week 0 (mg)	Week 6	Week 12, 24, 36 (mg)
<10	<70	1000	500 mg	500[a]
<10	≥70	1000	1000 mg	500[a]
10–14	<70	1000	No dose	500[a]
10–14	≥70	1000	500 mg	500[a]
≥14, <15	All	500	No dose	500[a]

[a] Dose to be administered if serum ferritin <100 mg/mL or serum ferritin 100–300 ng/mL with transferrin saturation <20%

[Fig. 14: Treatment dosing scheme used in the CONFIRM-HF study][56]

Treatment with ferric carboxymaltose therefore resulted in a sustainable improvement in symptoms, quality of life and functional capacity, along with a reduction in the number of hospitalisations for worsening heart failure. The incidence of adverse events and number of deaths were similar.

The beneficial effects were encountered as quickly as 4 weeks after initiation of intravenous iron supplementation.

These findings apply only to patients with heart failure with reduced ejection fraction (HFrEF). The effects of treating iron-deficient patients with HFmrEF and HFpEF are not known. Furthermore, the long-term safety of iron therapy in heart failure is unknown. The safety of IV iron in patients with heart failure with a haemoglobin of >15 g/dL also remains to be elucidated.

A search for potentially reversible and treatable causes (such as gastrointestinal bleeding) of iron deficiency must be made in all patients.

Parenteral iron supplementation:

In the general population with iron deficiency anaemia, historically the first parenteral compounds used for iron repletion were administered as iron oxyhydroxide complexes. This produced a significant amount of non-transferrin-bound iron, which in turn resulted in higher oxidative stress. Several adverse effects occurred as a result, and these included nausea, vomiting and hypotension. This problem was corrected after compounds were introduced that contained the iron moiety in a core surrounded by a shell of carbohydrate.

The parenteral formulations that have been approved for therapeutic use include

- Ferric sorbitol
- Iron dextrans (high and low-molecular weight dextran)
- Iron polymaltose

- Iron sucrose
- Ferric gluconate
- Ferric carboxymaltose
- Iron isomaltoside 1000
- Ferumoxytol

Five of these compounds were formally investigated for use in patients who had heart failure, with the weight of the evidence in favour of iron sucrose and ferric carboxymaltose. Three other compounds (iron dextran, ferric gluconate and iron isomaltoside 1000) were examined in only a small single-centre study.[56]

Current recommendations:

Based on the existing evidence, the 2016 European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic heart failure recommend that IV iron therapy should be considered in all symptomatic patients who have heart failure with reduced ejection, along with absolute or functional iron deficiency (defined as serum ferritin <100 µg/L or a ferritin between 100-299 µg/L with a transferrin saturation <20%).

(Class of recommendations IIa, level of evidence A)[3]

	High-molecular weight iron dextran	Low-molecular weight iron dextran	Ferric carboxymaltose	Iron sucrose	Ferric gluconate	Iron isomaltoside 1000	Ferumoxytol	Iron polymaltose
Trade name	DexFerrum	INFeD	Ferinject, injectafer	Venofer	Ferrlecit	Monofer	Feraheme	Ferrosig
Carbohydrate shell	Complex branched glucan	Complex branched glucan	Carboxymaltose (branched polysaccharide)	Sucrose (disaccharide)	Gluconate (monosaccharide)	Linear chemical structure of average 5.2 glucose units	Polyglucose sorbitol carboxymethyl ether	Dextrin
Molecular weight by manufacturer, Dalton $\times 10^3$	265	<165	150	34–60	289–444	150	750	462
Dosage used for the PK characteristics, mg Fe	Not stated	500–2000	100/1000	100	125	100/200	316	100
Terminal half-life, h	9.4–87.4, average 58.9	5.20	7.4/9.4	5.3	1.42	20.8–23.5	14.7	22.4
Test dose required	Yes	Yes	No	No	No	No	No	No

PK pharmacodynamic

[Fig. 15: Available intravenous iron formulations][56]

Ongoing research:

It is as of yet unknown if treatment with intravenous iron can reduce mortality in patients with heart failure, and hence large-scale mortality-morbidity trials are currently being undertaken for both acute heart failure (AFFIRM-AHF trial) and chronic heart failure (IRONMEN trial).

Materials and Methods

MATERIALS AND METHODS

Study centre:

Institute of Internal Medicine, Rajiv Gandhi Government General Hospital, Madras Medical College, Chennai.

Ethical Committee approval:

Obtained.

Study duration:

Six months.

Study design:

Case-control study.

Sample size:

100 (comprising 50 cases and 50 age-matched controls).

Inclusion criteria for cases:

1. Patients with typical symptoms and signs of heart failure

   (OR)

   Asymptomatic patients who are on anti-failure pharmacotherapy for a previous episode of symptomatic heart failure.
2. Ejection fraction <40% on echocardiography.
3. Age >40 years.

Exclusion criteria for cases:

1. Patients with specific aetiologies for heart failure (eg, valvular heart disease, congenital heart disease).
2. Hospitalisation, acute coronary syndrome or coronary revascularization over the last 30 days.
3. Any acute/chronic illness other than heart failure that may influence iron metabolism (including known malignancy, active bleeding, infection, severe renal disease requiring dialysis, haematological diseases).
4. Treatment for anaemia and/or iron deficiency (blood transfusions, erythropoietin therapy, iron supplements) within the last 6 months.

Inclusion criteria for controls:

1. Asymptomatic individuals with no evidence of heart failure based on history and clinical examination.
2. Ejection fraction >50% on echocardiography.
3. Age >40 years.

Exclusion criteria for controls:

1. Any acute/chronic illness other than heart failure that may influence iron metabolism (including known malignancy, active bleeding,

infection, severe renal disease requiring dialysis, haematological diseases).

2. Treatment for anaemia and/or iron deficiency (blood transfusions, erythropoietin therapy, iron supplements) within the last 6 months.

Data collection:

Participants for this study were enrolled by a process of simple random sampling of patients attending the out-patient clinic of the Department of Cardiology, Rajiv Gandhi Government General Hospital, Madras Medical College, who met the pre-specified inclusion and exclusion criteria.

Controls were chosen from apparently healthy family members of patients admitted in the Institute of Internal Medicine, Rajiv Gandhi Government General Hospital, Madras Medical College, after ensuring that they too met the pre-specified inclusion and exclusion criteria. Age-matched controls were chosen, such that number of cases and number of controls below the age of 60 were equal. Similarly, there were an equal number of cases and controls who were aged over 60 years.

Informed consent was obtained from all participants. They were subjected to thorough history-taking and clinical examination.

Fresh peripheral venous blood samples were collected in ethylene diaminetetraacetic acid (EDTA) and serum tubes and sent for analysis. A complete haemogram with red cell indices was performed.

Serum ferritin was measured using a fully automated bidirectionally interfaced chemi luminescent immuno assay. Serum iron was measured by the ferrozine method without deproteinisation. Total iron binding capacity (TIBC) was measured by a spectrophotometric assay. Transferrin saturation was calculated as 100 x serum iron / TIBC.

Participants were subjected to a comprehensive transthoracic Doppler echocardiography, performed using standardised equipment. The biplane method of disks was used to evaluate left ventricular ejection fraction.

Definition of anaemia:

The WHO definition of anaemia was used. Accordingly, women with a haemoglobin concentration <12.0 g/dL and men with a haemoglobin concentration <13.0 g/dL were deemed to be anaemic.[42]

Definition of iron deficiency:

The definition of iron deficiency used in several recent clinical studies[25, 26, 41, 46, 50, 53, 54, 57] was taken for this analysis. Iron deficiency was defined as a serum ferritin <100 µg/L or a ferritin between 100-299 µg/L with a transferrin saturation <20%.

Observations and Results

OBSERVATIONS AND RESULTS

AGE DISTRIBUTION IN CASES AND CONTROLS:

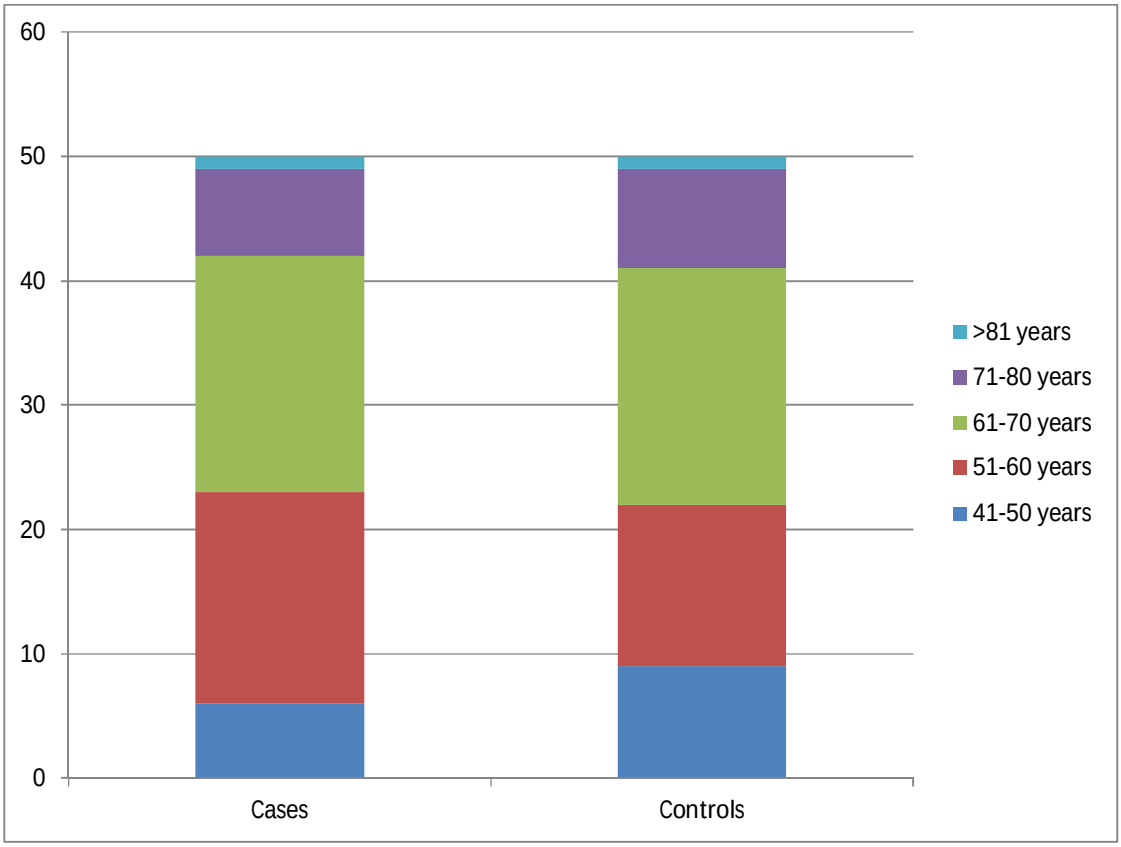
Age in years	Cases	Controls	Total
41-50 years	6	10	16
51-60 years	17	13	30
61-70 years	19	18	37
71-80 years	7	8	15
>81 years	1	1	2
Total	50	50	100

[Table 1: Age distribution of cases and controls]

- Total number of cases = 50
- Total number of controls = 50

The controls were age-matched to the cases.

- Number of cases below age of 60 = 23
- Number of controls below age of 60 = 23
- Number of cases above age of 60 = 27
- Number of controls above age of 60 = 27
- Percentage of patients below age of 60 = 46%
- Percentage of patients above age of 60 = 54%



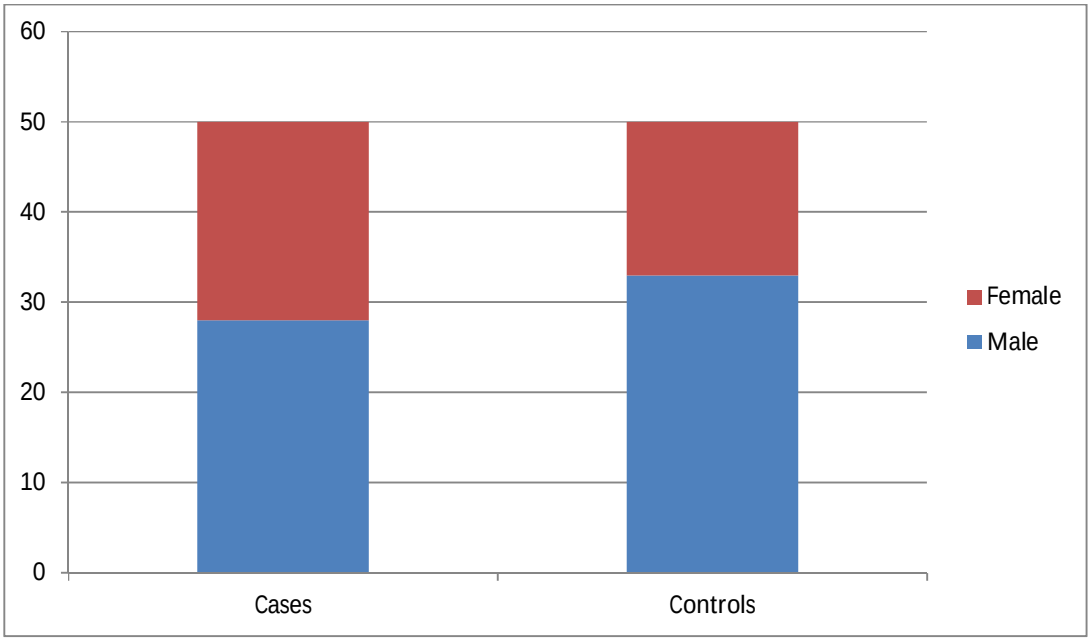
[Fig. 16: Age distribution of cases and controls]

SEX DISTRIBUTION IN CASES AND CONTROLS:

Sex	Cases	Controls	Total
Male	28	33	61
Female	22	17	39
Total	50	50	100

[Table 2: Sex distribution in cases and controls]

- Percentage of cases who are male = 56%
- Percentage of cases who are female = 44%
- Percentage of controls who are male = 66%
- Percentage of controls who are female = 34%



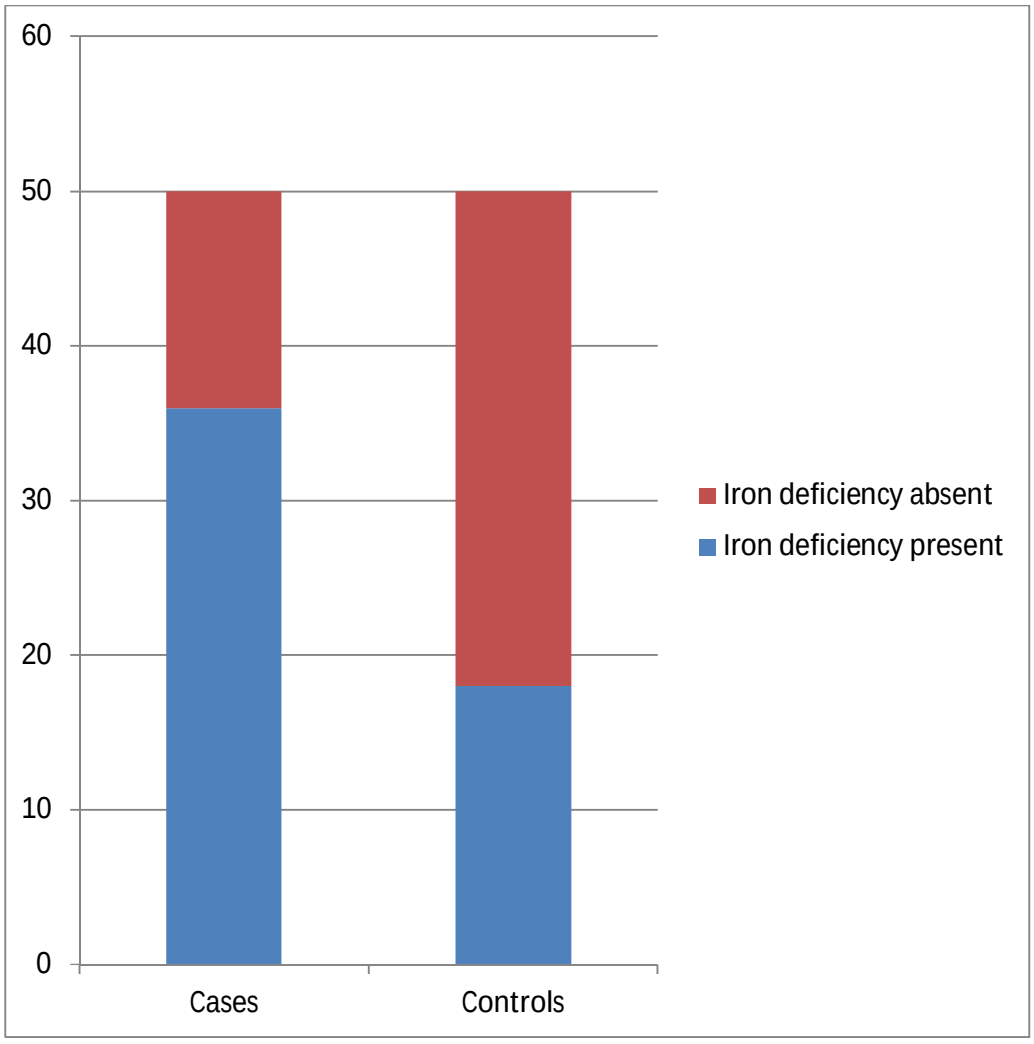
[Fig 17: Sex distribution of cases and controls]

IRON DEFICIENCY IN CASES AND CONTROLS:

	Cases	Controls	Total
Iron deficient	36	18	54
No iron deficiency	14	32	46
Total	50	50	100

[Table 3: Iron deficiency in cases and controls]

- Prevalence of iron deficiency in cases = 72%
- Prevalence of iron deficiency in controls = 36%
- Odds ratio = 4.57
- The Null Hypothesis in this case is “There is no significant difference in the prevalence of iron deficiency, between cases and controls”
- Degree of freedom = 1
- Chi-square statistic = 13.043
- P-value = 0.0003
- Thus the Null Hypothesis is rejected.



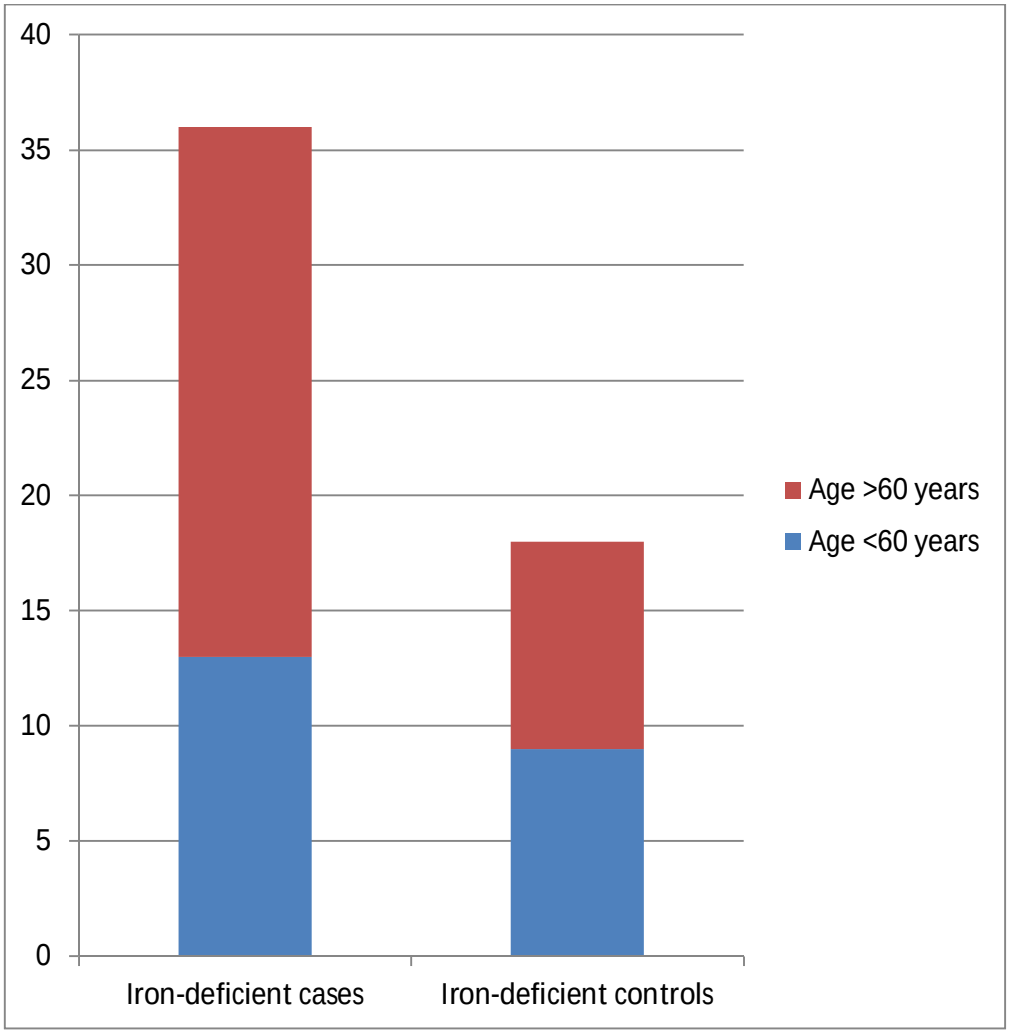
[Fig. 18: Iron deficiency in cases and controls]

EFFECT OF AGE ON IRON DEFICIENCY:

Age	Iron deficient cases	Iron deficient controls	Total
Age <60 years	13	9	22
Age >60 years	23	9	32
Total	36	18	54

[Table 4: Effect of age on iron deficiency]

- Percentage of cases with iron deficiency aged <60 years = 36.1%
- Percentage of cases with iron deficiency aged >60 years = 63.9%
- Percentage of controls with iron deficiency aged <60 years = 50%
- Percentage of controls with iron deficiency aged >60 years = 50%
- The Null Hypothesis in this case is "There is no significant effect of age on the prevalence of iron deficiency, among cases and controls"
- Degree of freedom = 1
- Chi-square statistic = 0.959
- P-value = 0.327
- Thus the Null Hypothesis is true.



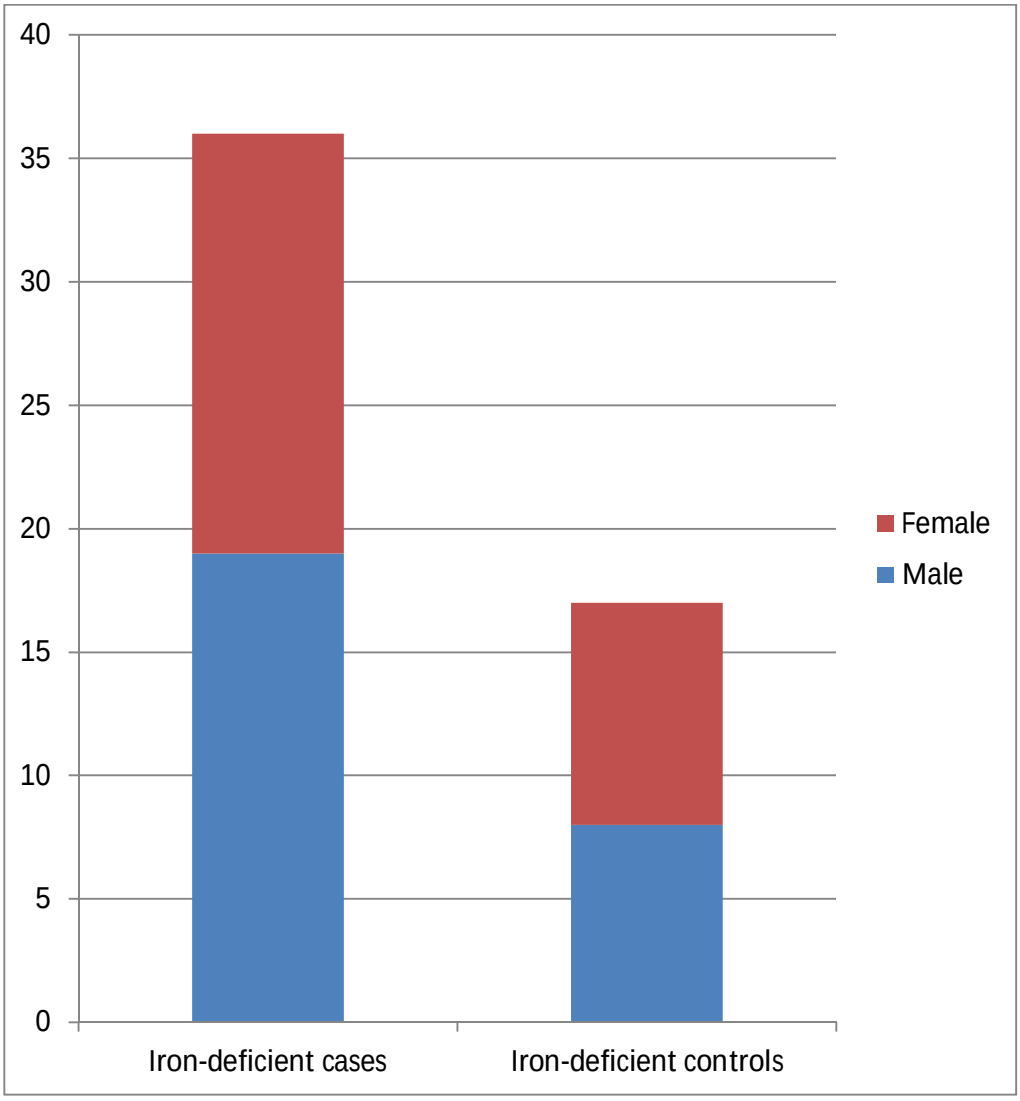
[Fig 19: Effect of age on iron deficiency]

EFFECT OF SEX ON IRON DEFICIENCY:

Sex	Iron-deficient cases	Iron-deficient controls	Total
Male	19	8	27
Female	17	10	27
Total	36	18	54

[Table 5: Effect of sex on iron deficiency]

- Percentage of cases with iron deficiency who are male = 52.8%
- Percentage of cases with iron deficiency who are female = 47.2%
- Percentage of controls with iron deficiency who are male = 44.4%
- Percentage of controls with iron deficiency who are female = 55.6%
- The Null Hypothesis in this case is "There is no significant effect of sex on the prevalence of iron deficiency, among cases and controls"
- Degree of freedom = 1
- Chi-square statistic = 0.333
- P-value = 0.5638
- Thus the Null Hypothesis is true.



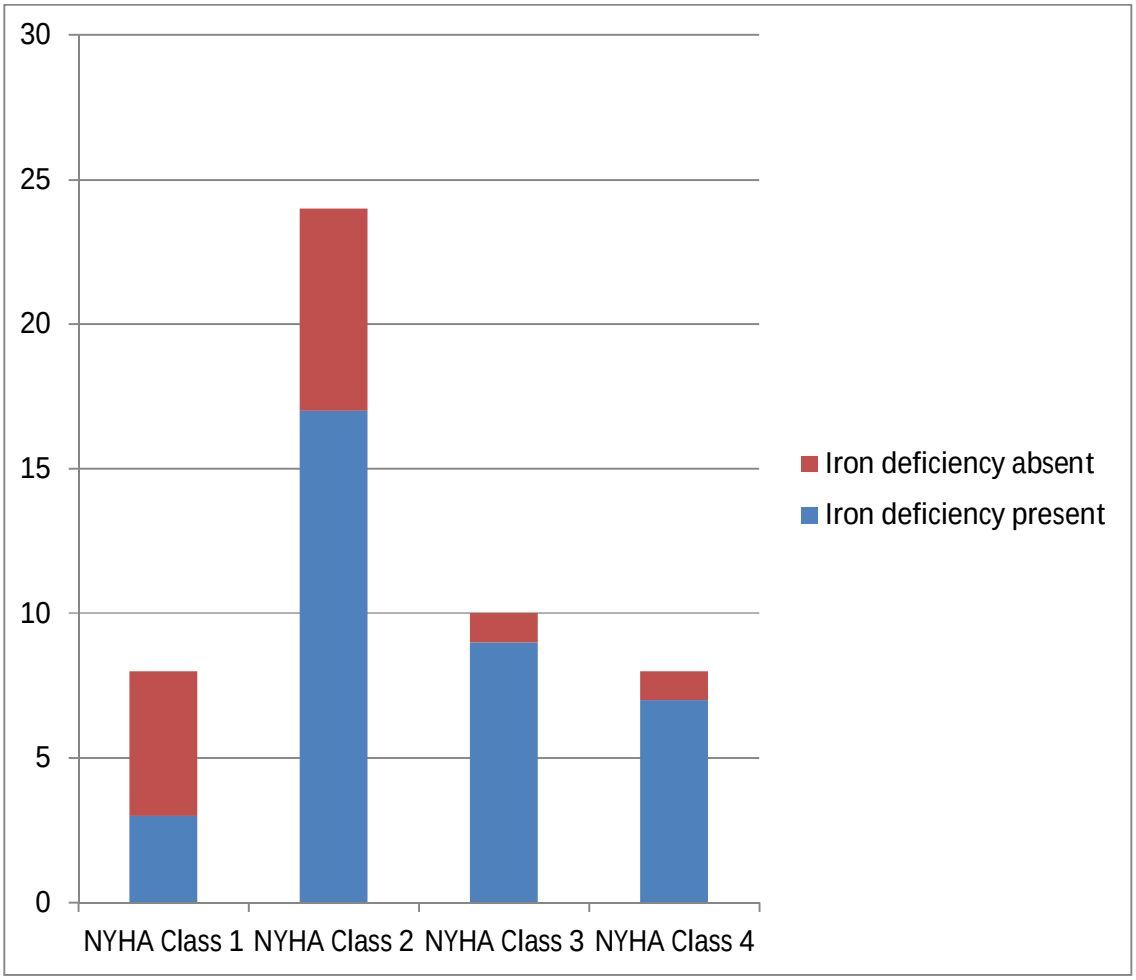
[Fig. 20: Effect of sex on iron deficiency]

DISTRIBUTION OF IRON DEFICIENCY BY NYHA CLASSIFICATION:

NYHA classification	Iron deficiency present	Iron deficiency absent	Total
Class 1	3	5	8
Class 2	17	7	24
Class 3	9	1	10
Class 4	7	1	8
Total	36	14	50

[Table 6: Distribution of iron deficiency by NYHA classification]

- Percentage of iron deficiency in NYHA Class 1 = 37.5%
- Percentage of iron deficiency in NYHA Class 2 = 70.8%
- Percentage of iron deficiency in NYHA Class 3 = 90%
- Percentage of iron deficiency in NYHA Class 4 = 87.5%
- The Null Hypothesis in this case is "There is no significant effect of NYHA class on the presence or absence iron deficiency"
- Degree of freedom = 3
- Chi-square statistic = 7.3
- P-value = 0.06
- Thus the Null Hypothesis is true.



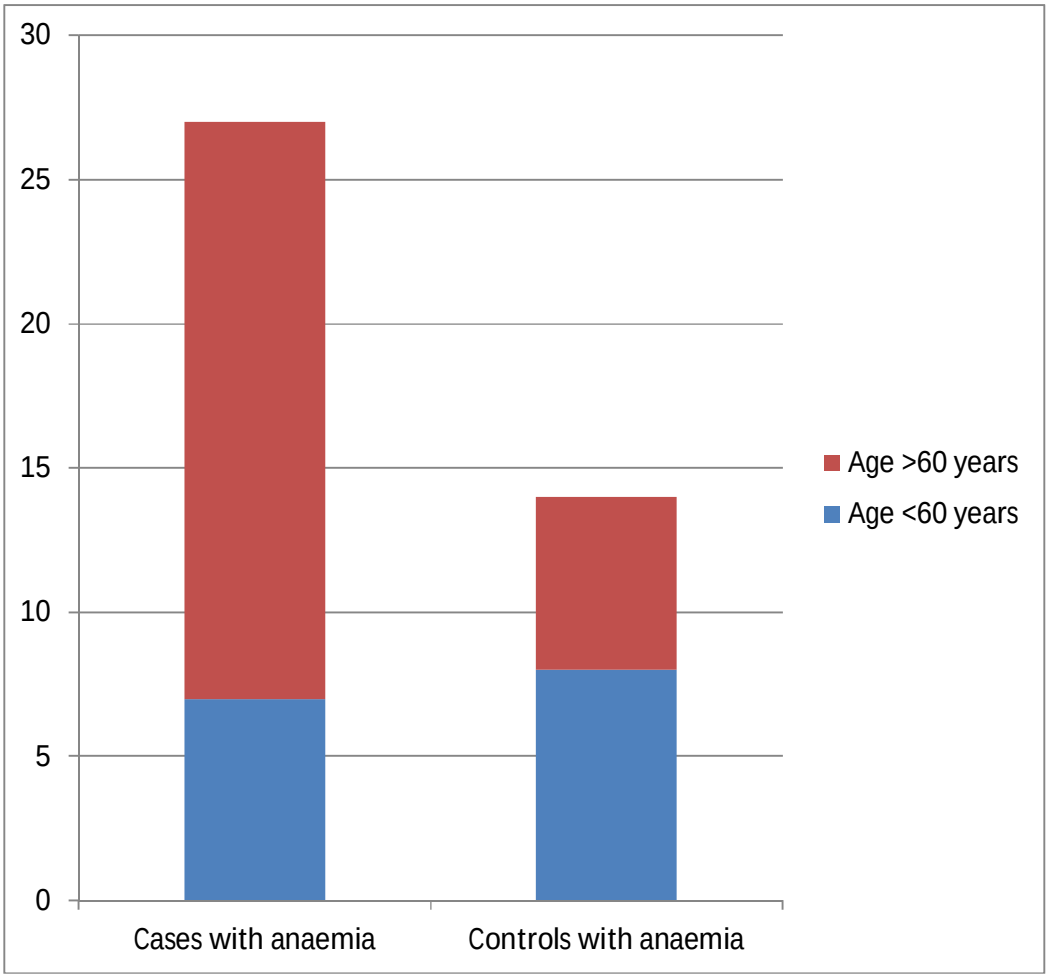
[Fig. 21: Distribution of iron deficiency by NYHA classification]

EFFECT OF AGE ON ANAEMIA:

Age	Cases with anaemia	Controls with anaemia	Total
Age <60 years	7	8	15
Age >60 years	20	6	26
Total	27	14	41

[Table 7: Effect of age on anaemia]

- Percentage of cases with anaemia aged <60 years = 25.9%
- Percentage of cases with anaemia aged >60 years = 74.1%
- Percentage of controls with anaemia aged <60 years = 57.1%
- Percentage of controls with anaemia aged >60 years = 42.9%
- The Null Hypothesis in this case is "There is no significant effect of age on the prevalence of anaemia, among cases and controls"
- Degree of freedom = 1
- Chi-square statistic = 3.873
- P-value = 0.049
- Thus, the Null Hypothesis is rejected.



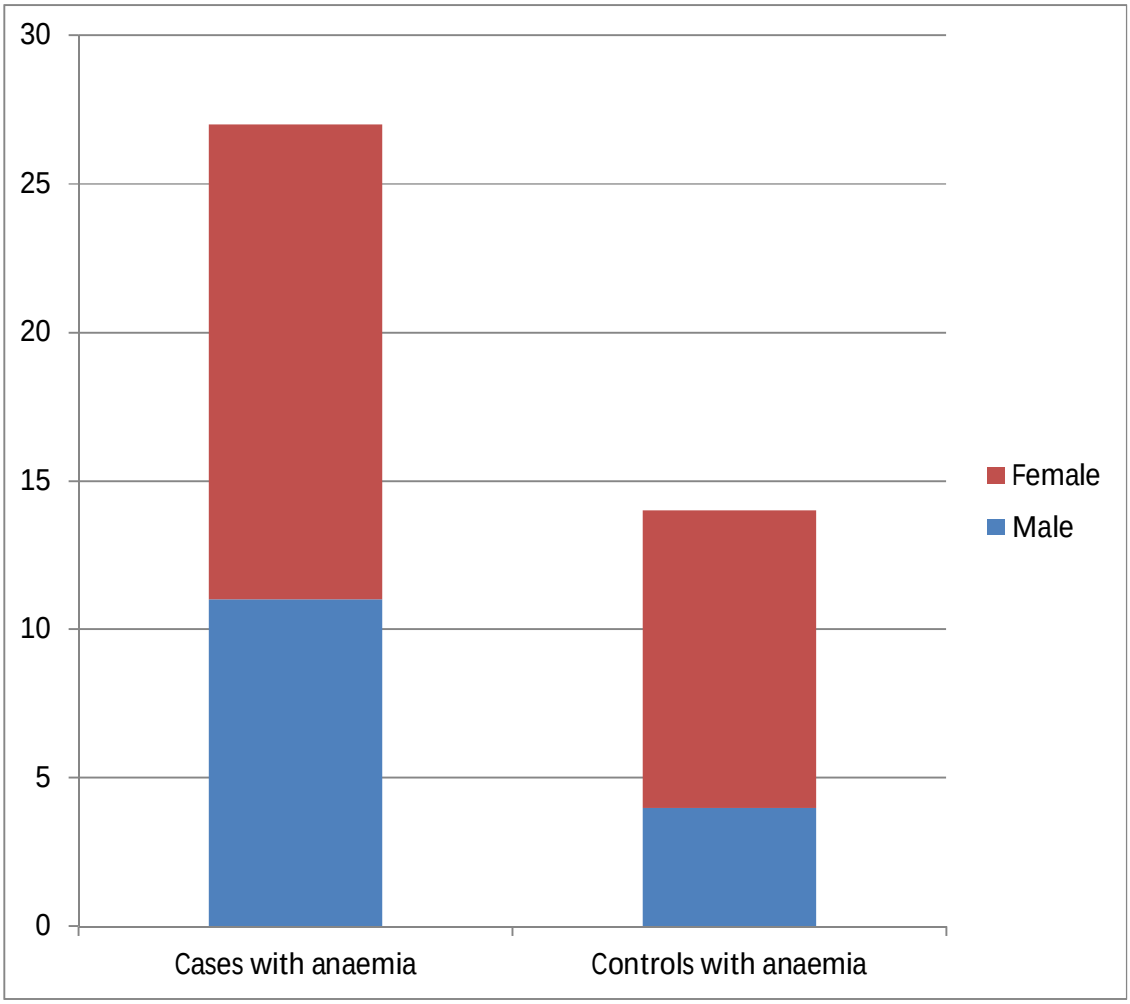
[Fig. 22: Effect of age on anaemia]

EFFECT OF SEX ON ANAEMIA:

Sex	Cases with anaemia	Controls with anaemia	Total
Male	11	4	15
Female	16	10	26
Total	27	14	41

[Table 8: Effect of sex on anaemia]

- Percentage of cases with anaemia who are male = 40.7%
- Percentage of cases with anaemia who are female = 59.3%
- Percentage of controls with anaemia who are male = 28.6%
- Percentage of controls with anaemia who are female = 71.4%
- The Null Hypothesis in this case is “There is no significant effect of sex on the prevalence of anaemia, among cases and controls”
- Degree of freedom = 1
- Chi-square statistic = 0.588
- P-value = 0.443
- Thus, the Null Hypothesis is true.



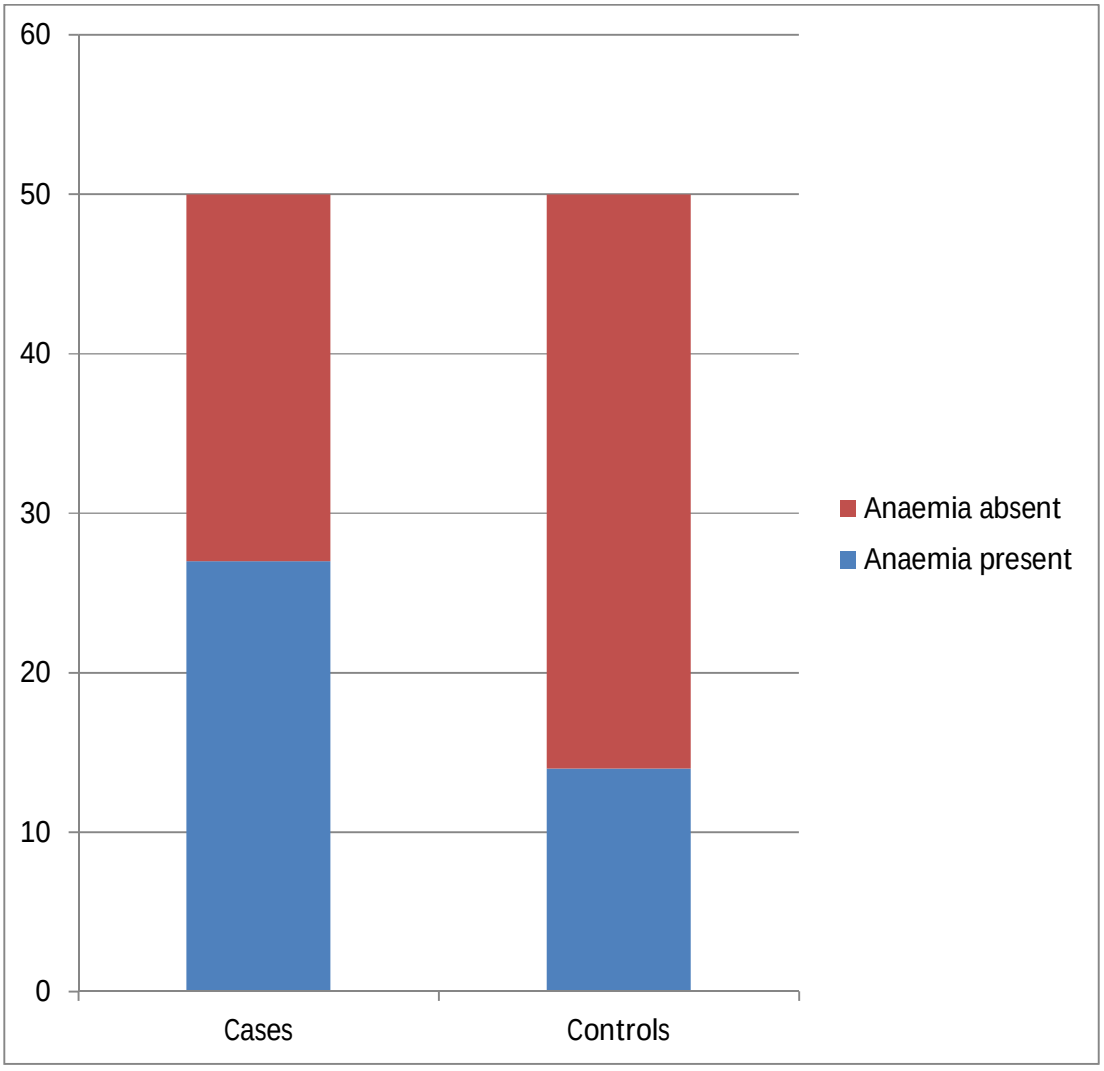
[Fig. 23: Effect of sex on anaemia]

ANAEMIA IN CASES AND CONTROLS:

	Cases	Controls	Total
Anaemia present	27	14	41
Anaemia absent	23	36	59
Total	50	50	100

[Table 9: Anaemia in cases and controls]

- Prevalence of anaemia in subjects in cases (with heart failure) = 54%
- Prevalence of anaemia in subjects in controls (without heart failure) = 28%
- Odds ratio = 3.01
- The Null Hypothesis in this case is “There is no significant difference in the prevalence of anaemia, between cases and controls”
- Degree of freedom = 1
- Chi-square statistic = 6.986
- P-value = 0.008
- Thus, the Null Hypothesis is rejected.



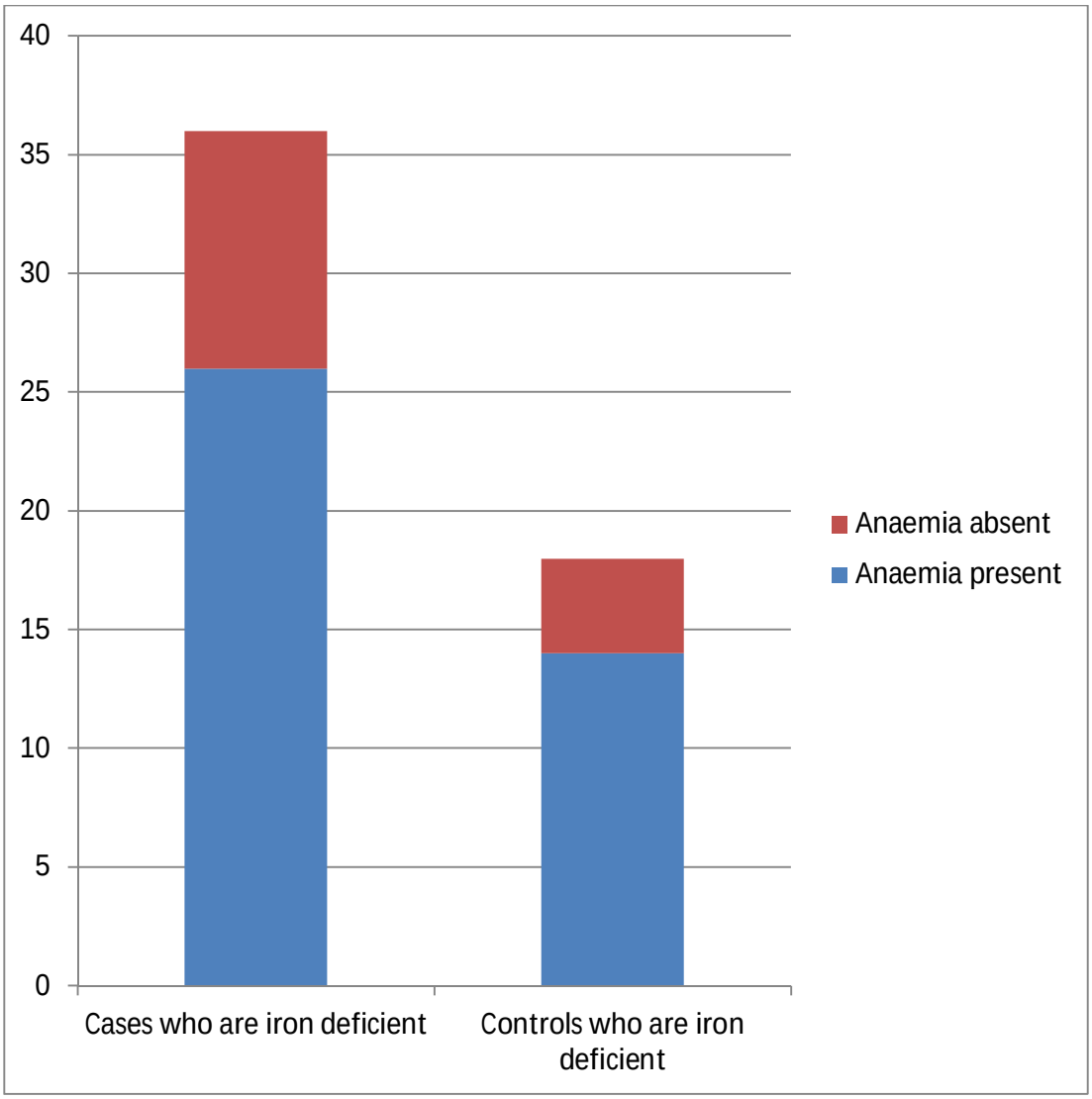
[Fig. 24: Anaemia in cases and controls]

FREQUENCY OF ANAEMIA IN PATIENTS WHO ARE IRON DEFICIENT:

	Cases who are iron deficient	Controls who are iron deficient	Total
Anaemia present	26	14	40
Anaemia absent	10	4	14
Total	36	18	54

[Table 10: Frequency of anaemia in patients who are iron deficient]

- Prevalence of anaemia in cases who are iron deficient = 72.2%
- Prevalence of anaemia in controls who are iron deficient = 77.7%
- The Null Hypothesis in this case is "There is no significant effect of the presence or absence of anaemia on the prevalence of iron deficiency among cases and controls"
- Degree of freedom = 1
- Chi-square statistic = 0.193
- P-value = 0.6604
- Thus the Null Hypothesis is true.



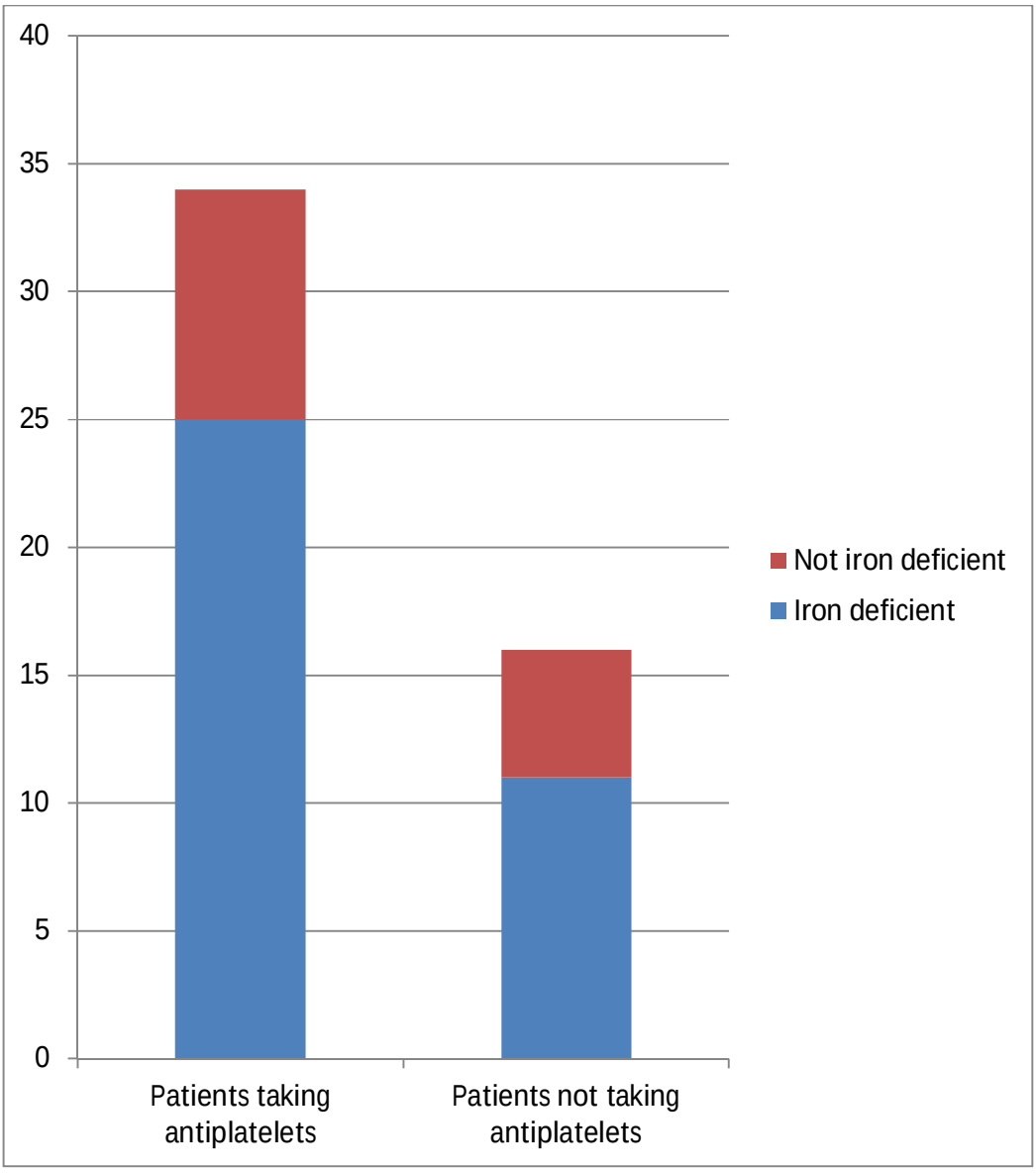
[Table 25: Frequency of anaemia in patients who are iron deficient]

EFFECT OF ANTIPLATELETS ON IRON DEFICIENCY AMONG CASES:

	Iron deficiency present	**Iron deficiency absent**	**Total**
Taking antiplatelet agents	25	9	34
Not taking antiplatelet agents	11	5	16
Total	36	14	50

[Table 11: Effect of antiplatelets on iron deficiency among cases]

- Prevalence of iron deficiency in patients taking antiplatelets = 73.5%
- Prevalence of iron deficiency in patients not taking antiplatelets = 68.75%
- The Null Hypothesis in this case is "The use of antiplatelet agents has no significant effect on the presence or absence of iron deficiency"
- Degree of freedom = 1
- Chi-square = 0.123
- P-value = 0.7258
- Thus the Null Hypothesis is true.



[Fig. 26: Effect of antiplatelets on iron deficiency among cases]

Discussion

DISCUSSION

For this study, 50 patients with heart failure with reduced ejection fraction (HFrEF; left ventricular ejection fraction <40%) who met the pre-specified inclusion and exclusion criteria were chosen. For comparison, 50 controls were chosen who were age-matched to the cases, and had no clinical or echocardiographic evidence of heart failure, who met the pre-specified inclusion and exclusion criteria, were also included in the study. A detailed history was taken and clinical examination was performed. Peripheral venous blood was drawn, and a complete haemogram along with iron studies (iron, ferritin, transferrin saturation and total iron binding capacity) were performed.

Age distribution of cases and controls:

Patients aged less than 40 years who had heart failure with reduced ejection fraction were excluded from the study. This was done as an attempt to exclude patients who had specific aetiologies for heart failure, including genetic cardiomyopathies and congenital heart diseases.

The patients enrolled as cases in this study had an age range from 41 to 83 years, with a mean age of 61.13 years (mean age among cases 61.6 years; mean age among controls 60.7 years) and a median age of 62 years.

It was ensured during patient selection that there were an equal number of cases and controls both below and above the age of 60, so as to allow comparison between the two groups. Thus the total number of patients aged less than 60 were 46 (23 cases, 23 controls) and the total number of patients aged over 60 were 54 (27 cases, 27 controls). In other words, 46% of the study population was below the age of 60, and the other 54% was above the age of 60.

Sex distribution among cases and controls:

In this study, there were a total of 61 male subjects (28 cases and 33 controls), and a total of 39 female subjects (22 cases and 17 controls). The percentage of male subjects is 56% among cases and 66% among controls, while the percentage of female subjects is 44% among cases and 34% among controls.

Iron deficiency in cases and controls:

The total number of iron deficient individuals was 54 (36 cases and 18 controls). The percentage of iron deficient individuals was 72% among the cases, and 36% among the controls. A chi-squared test performed on this data produced a P-value of 0.0003 which was statistically significant, suggesting that the prevalence of iron deficiency was higher among patients

with heart failure with reduced ejection fraction, as compared to normal individuals.

The odds ratio (cross-product ratio) was calculated to be 4.57, indicating that patients with heart failure with reduced ejection fraction were 4.57 times more likely to be iron deficient, as compared with normal individuals.

Effect of age on iron deficiency:

Among patients with heart failure who were iron deficient (n = 36), 13 patients (36.1%) were below the age of 60, and 23 patients (63.9%) were above the age of 60. Among controls who were iron deficient (n = 18), 9 patients were aged less than 60 years (50%), and similarly 9 patients were aged more than 60 years (50%). These variations were not found to be statistically significant. (P-value 0.327)

Effect of sex on iron deficiency:

Among patients with heart failure who were iron deficient (n = 36), 19 patients (52.8%) were male and 17 patients (47.2%) were female. Among controls who were iron deficient (n = 18), 8 patients (44.4%) were male and 10 patients (55.6%) were female. These variations were not found to be statistically significant. (P- value 0.5638)

Distribution of iron deficiency by NYHA classification:

Among the patients who had heart failure with NYHA class 1 (n = 8), 3 patients were iron deficient (37.5%). Among patients who had heart failure with NYHA class 2 (n = 24), 17 patients were iron deficient (70.8%). Among patients who had heart failure with NYHA class 3 (n = 10), 9 patients were iron deficient (90%). Among patients who had heart failure with NYHA class 4 (n = 8), 7 patients were iron deficient (87.5%).

Thus as patients advance from NYHA Class 1 through NYHA Class 4, there appears to be an increase in the proportion of patients with iron deficiency. However, these variations were not found to be statistically significant (P-value 0.06)

Effect of age on anaemia:

Among patients with heart failure who had anaemia (n = 27), 7 patients (25.9%) were aged below 60 years, and 20 patients (74.1%) were aged above 60 years. Among individuals in the control group who had anaemia (n = 14), 8 patients (57.1%) were aged below 60 years, and 6 patients (42.9%) were aged above 60 years. These variations were found to be statistically significant (P-value 0.049)

Effect of sex on anaemia:

Among patients with heart failure who had anaemia (n = 27), 11 patients (40.7%) were male and 16 patients (59.3%) were female. Among individuals in the control group who had anaemia (n = 14), 4 patients (28.6%) were male and 10 patients (71.4%) were female. These variations were not statistically significant (P-value 0.443)

Anaemia in cases and controls:

Among the 50 patients with heart failure (cases) enrolled in this study, it was found that 27 patients were anaemic (prevalence of 54%). Similarly, among the 50 individuals without heart failure (controls) enrolled in this study, it was found that 14 individuals were anaemic (prevalence 28%). A chi-squared test was applied as a test of significance, and this yielded a P-value of 0.008, indicating that the data is statistically significant.

The odds-ratio (cross-product ratio) was calculated to be 3.01. This indicated that patients with heart failure had a 3.01 greater risk of anaemia, as compared to patients without heart failure.

Frequency of anaemia in patients who are iron deficient:

Among the patients with heart failure who were iron deficient (n = 36), it was found that anaemia was present in 26 patients (72.2%). Similarly, among controls who were iron deficient (n = 18), it was found that anaemia

was present in 14 individuals (77.7%). This variation was not found to be statistically significant (P-value 0.6604).

Effect of antiplatelets on iron deficiency among cases:

Among the patients with heart failure who were iron deficient (n = 36), it was noted that 25 patients (73.5%) were taking antiplatelet agents (aspirin or clopidogrel) for at least 30 days. Among patients with heart failure who were not iron deficient (n = 14), it was noted that 9 patients (68.5%) were taking antiplatelet agents for at least 30 days. This variation was not found to be statistically significant. Thus it appears that the use of antiplatelet agents such as aspirin and clopidogrel does not increase the risk of iron deficiency in patients with heart failure.

Effect of anticoagulants on iron deficiency:

Among the 100 participants in the study, only one patient was taking an anticoagulant (acitrom) for the last 30 days, and hence no inferences can be drawn regarding the increased risk of iron deficiency or anaemia during chronic anticoagulant use.

The one patient who was taking acitrom did not have iron deficiency.

Comparison of results with other studies:

Tee Joo Yeo et al[25]:

A study titled “Iron deficiency in a multi-ethnic Asian population with and without heart failure: prevalence, clinical correlates, functional significance and prognosis” was published in the European Journal of Heart Failure in 2014.

Patients with compensated heart failure were enrolled, irrespective of the presence of reduced or preserved ejection fraction. The European Society of Cardiology (ESC) guidelines and Framingham criteria were used to make the diagnosis. Patients with severe comorbidities and specific aetiologies to heart failure were excluded, as such patients were presumed to have a different natural history when compared with a patient with “typical” heart failure.

Controls were selected from adults aged over 55 years who were living in the general community in Singapore. A total of 751 cases and 601 controls were enrolled.

The heart failure group had an average age of 62.0 ± 12.2 years. Females comprised 25% of the patients, with males making up the remaining 75%. Only 10.2% of the population were Indian. The control group was younger, and had 49.8% of male patients.

Iron deficiency was seen in 61.4% of patients with heart failure and in 39.3% of controls (P <0.001) and this difference remained significant after adjusting for race, age, body size, gender and comorbidities that included diabetes, hypertension and renal status. Functional iron deficiency was also found to be higher among patients with heart failure than the control group (odds ratio 3.5, P<0.001).

Iron deficiency prevalence was more in women compared with men in both cases (70.5% vs 58.6%, P = 0.004) and controls (57.9% vs 20.4%, P <0.001), even after adjusting for race, age, body mass index, alcohol and smoking status, and comorbidities (odds ratio for heart failure group 2.8, P <001; odds ratio for control group 5.8, P <0.001).

Among the different ethnic groups included (64.7% Chinese, 23.9% Malay and 10.2% Indian), the prevalence of iron deficiency was highest in Indian participants in both groups (P <0.001), and this difference persisted

even after adjustment for age, gender, smoking and alcohol status, body mass index, and comorbidities (P = 0.001).

The different factors found to be independently associated with iron deficiency in patients with heart failure were Indian race, female gender, larger body mass index, and decreased left ventricular ejection fraction. Within the control group, Indian participants and younger women were more likely to have iron deficiency.

Darlington O. Okonko et al[27]:

A study titled "Disordered iron homeostasis in chronic heart failure: Prevalence, predictors, and relation to anemia, exercise capacity and survival" was published in the Journal of the American College of Cardiology in 2011.

157 patients with heart failure with an ejection fraction of <45% were enrolled, along with 22 control subjects who had no known medical problems.

Patients and controls were similar to controls with respect to age and sex, but the cases had lower haemoglobin and higher creatinine levels. Iron deficiency was present in 68 patients (43%). This iron deficiency was not affected by the use of antiplatelet agents or anticoagulants, but was more

prevalent with higher NYHA functional class designations, lower haemoglobin, higher CRP and white counts, and beta blocker use.

Patients with heart failure had lower transferrin saturation, but higher serum iron, TIBC and soluble transferrin receptors compared with controls. Circulating deficits were found to be worse with greater NYHA functional class and greater anaemia.

The median ferritin levels were less in female patients and more in male patients. When patients were stratified by NYHA functional class, a progressive decline in ferritin levels was found from patients in class I or II, to patients in class III, and then to patients in class IV (analysis-of-variance $p = 0.04$).

When the FERRIC-HF criteria were used to define iron deficiency, it was found that 69% of heart failure patients were iron deficient, along with 78% of all anaemic patients and 65% of all non-anaemic patients. When analysed separately, functional iron deficiency was present in 10% of subjects and absolute iron deficiency in 59% of subjects.

Inês Rangel et al[26]:

A study titled “Iron deficiency status irrespective of anemia: A predictor of unfavorable outcome in chronic heart failure patients” was published in Cardiology in 2014.

127 patients with chronic stable heart failure with a left ventricular ejection fraction of <45% (as assessed by the echocardiographic planimetric Simpson method) were enrolled. 46 patients were diagnosed to be iron deficient, and this corresponds to a prevalence of 36% with a slight predominance in the anaemic group, which was nonsignificant ($p = 0.408$). 34 patients (74%) had iron deficiency without anaemia. Of the 46 patients with iron deficiency, 26 patients (57%) had absolute iron deficiency and the remaining 20 patients (43%) had functional iron deficiency.

No control group was taken, but anaemia was present in 22% of the general population. Patients with iron deficiency did not appear to have anaemia more frequently that those without iron deficiency.

Factors associated with iron deficiency were female sex, therapy with loop diuretics, right ventricular systolic dysfunction and plasma BNP levels >400 pg/mL. There were no other baseline characteristics that significantly differed between individuals with and without iron deficiency, and between patients with absolute and functional iron deficiency.

John G. F. Cleland et al[38]:

An original investigation was published in JAMA Cardiology in 2016, titled “Prevalence and Outcomes of Anemia and Hematinic Deficiencies in Patients with Chronic Heart Failure”.

4456 were enrolled, and the median age was 73 years (65-79),1760 participants were women (39.5%), 1791 participants had left ventricular systolic dysfunction (40.2%).

Overall the patients with anaemia numbered 1237 (27.8%), and among this 643 had mild anaemia (14.4%), 354 had moderate anaemia (7.9%) and 240 had marked anaemia (5.4%). Patients with left ventricular systolic dysfunction had a higher prevalence of anaemia (597, 33.3%).

A strong association was noted between the prevalence of anaemia and age, especially in men. However, only a weak association was noted between anaemia and sex.

3545 patients had a measurement of serum iron (79.6%) and this was less than 45 µg/dL in 497 patients (14.0%) and less than 47 µg/dL in 1296 patients (36.6%). Patients who had anaemia were also much more likely to have decreased serum iron levels, irrespective of cardiac phenotype. Serum iron was found to be highly correlated with measurements of transferrin

saturation ($P < 0.001$), but only weakly correlated with measurements of ferritin ($P = 0.01$)

3373 patients had a measurement of serum ferritin (75.5%) and this was lower than 30 ng/mL in 478 patients (10.7%) and lower than 100 ng/mL in 1951 patients (43.8%). Despite having a higher prevalence of anaemia, it was noted that patients with left ventricular systolic dysfunction were less likely to have a decreased serum ferritin concentration when compared with patients without heart failure.

Several patients were found to have a serum ferritin concentration of less than 100 ng/mL, yet they did not have anaemia. The prevalence of anaemia bore a poor relationship with serum ferritin.

Summary of Results

SUMMARY OF RESULTS

1. Prevalence of iron deficiency among patients with heart failure was 72% and among normal controls was 36%. This was statistically significant, and patients with heart failure were 4.57 times more likely to be iron deficient than those without heart failure.
2. Prevalence of anaemia among patients with heart failure was 54% and among normal controls was 28%. This was statistically significant, and patients with heart failure were 3.01 times more likely to be anaemic than those without heart failure.
3. The use of antiplatelet agents such as aspirin and clopidogrel were not associated with a higher risk of iron deficiency.

Conclusion

CONCLUSION

This study showed that there is a large burden of iron deficiency and anaemia in patients with heart failure with reduced ejection fraction, with iron deficiency being more common. It has been proved in randomised controlled trials that correction of this iron deficiency, irrespective of the presence or absence of concomitant anaemia, improves quality of life. It may therefore be prudent to assess and use iron status as a therapeutic target in all patients with heart failure. This is especially true of a country like India where a significant proportion of the population is iron deficient.

Limitations of the study

LIMITATIONS OF THE STUDY

1. The sample size of 50 cases is small, and larger studies may be required for accuracy.
2. Iron supplementation and re-assessment for symptomatic improvement was not attempted.
3. Follow-up and evaluation of the effects of iron deficiency on mortality was not possible.

Bibliography

BIBLIOGRAPHY

1. Davis RC, Hobbs FDR, Lip GYH. A History and epidemiology. *BMJ : British Medical Journal*. 2000;320(7226):39-42.
2. Norman J.N. William Withering and the purple foxglove: a bicentennial tribute. *J Clin Pharmacol*. 1985;25:479–483.
3. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2016;37:2129-200.
4. Wang TJ. Natural history of asymptomatic left ventricular systolic dysfunction in the community. *Circulation* 2003;108:977–982.
5. The SOLVD Investigators. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *N Engl J Med* 1992;327:685–691.
6. McMurray JJ V. Clinical practice. Systolic heart failure. N Engl J Med 2010;3623:228–238.
7. Chen J, Normand S-LT, Wang Y, Krumholz HM. National and regional trends in heart failure hospitalization and mortality rates for Medicare beneficiaries, 1998–2008. *JAMA* 2011;306:1669–1678.

8. Dunlay SM, Redfield MM, Weston SA, Therneau TM, Hall Long K, Shah ND, Roger VL. Hospitalizations after heart failure diagnosis a community perspective. *J Am Coll Cardiol* 2009;54:1695–1702.
9. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJ V, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WHW, Tsai EJ, Wilkoff BL. 2013 ACCF/AHA Guideline for the Management of Heart Failure: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation* 2013;128:1810–1852.
10. Metra M, Ponikowski P, Dickstein K, McMurray JJ V, Gavazzi A, Bergh C-H, Fraser AG, Jaarsma T, Pitsis A, Mohacsi P, Bo¨hm M, Anker S, Dargie H, Brutsaert D, Komajda M. Advanced chronic heart failure: a position statement from the Study Group on Advanced Heart Failure of the Heart Failure Association of the European Society of Cardiology. *Eur J Heart Fail* 9:684–694.
11. Killip T 3rd, Kimball JT. Treatment of myocardial infarction in a coronary care unit. A two year experience with 250 patients. *Am J Cardiol* 1967;20:457–464.
12. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. *Heart* 2007;93:1137–1146.

13. Redfield MM, Jacobsen SJ, Burnett JC, Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA* 2003;289:194–202.
14. Bleumink GS, Knetsch AM, Sturkenboom MCJM, Straus SMJM, Hofman A, Deckers JW, Witteman JCM, Stricker BHC. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. *Eur Heart J England*; 2004;25:1614–1619.
15. Ceia F, Fonseca C, Mota T, Morais H, Matias F, De Sousa A, Oliveira AG. Prevalence of chronic heart failure in Southwestern Europe: the EPICA study. *Eur J Heart Fail* 2002;4:531–539.
16. van Riet EES, Hoes AW, Limburg A, Landman MAJ, van der Hoeven H, Rutten FH. Prevalence of unrecognized heart failure in older persons with shortness of breath on exertion. *Eur J Heart Fail* 2014;16:772–777.
17. Kelder JC, Cramer MJ, van Wijngaarden J, van Tooren R, Mosterd A, Moons KGM, Lammers JW, Cowie MR, Grobbee DE, Hoes AW. The diagnostic value of physical examination and additional testing in primary care patients with suspected heart failure. *Circulation* 2011;124:2865–2873.
18. Boonman-de Winter LJM, Rutten FH, Cramer MJ, Landman MJ, Zuithoff NPA, Liem AH, Hoes AW. Efficiently screening heart failure in patients with type 2 diabetes. *Eur J Heart Fail* 2015;17:187–195.
19. Rutten FH, Moons KGM, Cramer M-JM, Grobbee DE, Zuithoff NPA, Lammers J-WJ, Hoes AW. Recognising heart failure in elderly patients with

stable chronic obstructive pulmonary disease in primary care: cross sectional diagnostic study. *BMJ* 2005;331:1379.

20. Hawkins NM, Petrie MC, Jhund PS, ChalmersGW, Dunn FG, McMurray JJV. Heart failure and chronic obstructive pulmonary disease: diagnostic pitfalls and epidemiology. *Eur J Heart Fail* 2009;11:130–139.
21. Daniels LB, Clopton P, Bhalla V, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, Omland T, Storrow AB, Abraham WT, Wu AHB, Steg PG, Westheim A, Knudsen CW, Perez A, Kazanegra R, Herrmann HC, McCullough PA, Maisel AS. How obesity affects the cut-points for B-type natriuretic peptide in the diagnosis of acute heart failure. Results from the Breathing Not Properly Multinational Study. *Am Heart J* 2006;151:999–1005.
22. Schaufelberger M, Swedberg K, Koster M, Rosen M, Rosengren A. Decreasing one-year mortality and hospitalization rates for heart failure in Sweden; data from the Swedish Hospital Discharge Registry 1988 to 2000. *Eur Heart J* 2004;25:300–307.
23. Jankowska EA, Kasztura M, Sokolski M, Bronisz M, Nawrocka S, Oles´kowska-Florek W, Zymlin´ski R, Biegus J, Siwołowski P, Banasiak W, Anker SD, Filippatos G, Cleland JGF, Ponikowski P. Iron deficiency defined as depleted iron stores accompanied by unmet cellular iron requirements identifies patients at the highest risk of death after an episode of acute heart failure. *Eur Heart J* 2014;35:2468–2476.

24. Jankowska EA, Malyszko J, Ardehali H, Koc-Zorawska E, Banasiak W, von Haehling S, Macdougall IC, Weiss G, McMurray JJV, Anker SD, Gheorghiade M, Ponikowski P. Iron status in patients with chronic heart failure. *Eur Heart J* 2013;34:827–834.
25. Yeo TJ, Yeo PS, Ching-Chiew Wong R, Ong HY, Leong KT, Jaufeerally F, Sim D, Santhanakrishnan R, Lim SL, M MYC, Chai P, Low AF, Ling LH, Ng TP, Richards AM, Lam CS. Iron deficiency in a multi-ethnic Asian population with and without heart failure: prevalence, clinical correlates, functional significance and prognosis. *Eur J Heart Fail* 2014;16:1125–1132.
26. Rangel I, Goncalves A, de Sousa C, Leite S, Campelo M, Martins E, Amorim S, Moura B, Silva Cardoso J, Maciel MJ. Iron deficiency status irrespective of anemia: a predictor of unfavorable outcome in chronic heart failure patients. *Cardiology* 2014;128:320–326.
27. Okonko DO, Mandal AK, Missouris CG, Poole-Wilson PA. Disordered iron homeostasis in chronic heart failure: prevalence, predictors, and relation to anemia, exercise capacity, and survival. *J Am Coll Cardiol* 2011;58:1241–1251.
28. Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood* 2003;102:783–788.
29. Jankowska EA, von Haehling S, Anker SD, Macdougall IC, Ponikowski P. Iron deficiency and heart failure: diagnostic dilemmas and therapeutic perspectives. *Eur Heart J* 2013;34:816–829.

30. Chua AC, Graham RM, Trinder D, Olynyk JK. The regulation of cellular iron metabolism. *Crit Rev Clin Lab Sci* 2007;44:413–459.

31. Fitzsimons S, Doughty RN; Iron deficiency in patients with heart failure. *Eur Heart J Cardiovasc Pharmacother*. 2015 Jan;1(1):58-64.

32. Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood* 2003;101:2461–2463.

33. Suzuki T, Hanawa H, Jiao S, Ohno Y, Hayashi Y, Yoshida K, Kashimura T, Obata H, Minamino T. Inappropriate expression of hepcidin by liver congestion contributes to anemia and relative iron deficiency. *J Card Fail* 2014;20:268–277.

34. Cohen-Solal A, Leclercq C, Mebazaa A, De Groote P, Damy T, Isnard R, Galinier M. Diagnosis and treatment of iron deficiency in patients with heart failure: Expert position paper from French cardiologists. *Arch Cardiovasc Dis* 2014;107:563–571.

35. Ferraro S, Mozzi R, Panteghini M. Revaluating serum ferritin as a marker of body iron stores in the traceability era. *Clin Chem Lab Med* 2012;50:1911–1916.

36. Coenen JL, van Dieijen-Visser MP, van Pelt J, van Deursen CT, Fickers MM, van Wersch JW, Brombacher PJ. Measurements of serum ferritin used to predict concentrations of iron in bone marrow in anemia of chronic disease. *Clin Chem* 1991;37:560–563.

37. Ebner N, von Haehling S. Iron deficiency in heart failure: a practical guide. *Nutrients* 2013;5:3730–3739.

38. Cleland JGF, Zhang J, Pellicori P, Dicken B, Dierckx R, Shoaib A, Wong K, Rigby A, Goode K, Clark AL. Prevalence and outcomes of anemia and hematinic deficiencies in patients with chronic heart failure. *JAMA Cardiol.* 2016;1(5):539-547.

39. Leszek P, Sochanowicz B, SzperlM, Kolsut P, Brzoska K, PiotrowskiW, Rywik TM, Danko B, Polkowska-Motrenko H, Rozanski JM, Kruszewski M. Myocardial iron homeostasis in advanced chronic heart failure patients. *Int J Cardiol* 2012;159:47–52.

40. Braga F, Infusino I, Dolci A, Panteghini M. Soluble transferrin receptor in complicated anemia. *Clin Chim Acta* 2014;431:143–147.

41. Cohen-Solal A, Damy T, Terbah M, Kerebel S, Baguet JP, Hanon O, Zannad F, Laperche T, Leclercq C, Concas V, Duvillie L, Darne B, Anker S, Mebazaa A. High prevalence of iron deficiency in patients with acute decompensated heart failure. *Eur J Heart Fail* 2014;16:984–991.

42. Blanc B, Finch CA, Hallberg L, et al. Nutritional anaemias. Report of a WHO Scientific Group. WHO Tech Rep Ser. 1968;405: 1-40.

43. Rangel I, Goncalves A, de Sousa C, Leite S, Campelo M, Martins E, Amorim S, Moura B, Silva Cardoso J, Maciel MJ. Iron deficiency status irrespective of anemia: a predictor of unfavorable outcome in chronic heart failure patients. *Cardiology* 2014;128:320–326.

44. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005;352:1011–1023.

45. Weber CS, Beck-da-Silva L, Goldraich LA, Biolo A, Clausell N. Anemia in heart failure: association of hepcidin levels to iron deficiency in stable outpatients. *Acta Haematol* 2013;129:55–61.

46. Jankowska EA, Rozentryt P, Witkowska A, Nowak J, Hartmann O, Ponikowska B, Borodulin-Nadzieja L, Banasiak W, Polonski L, Filippatos G, McMurray JJ, Anker SD, Ponikowski P. Iron deficiency: an ominous sign in patients with systolic chronic heart failure. *Eur Heart J* 2010;31:1872–1880.

47. Maeder MT, Khammy O, dos Remedios C, Kaye DM. Myocardial and systemic iron depletion in heart failure implications for anemia accompanying heart failure. *J Am Coll Cardiol* 2011;58:474–480.

48. Dong F, Zhang X, Culver B, Chew HG Jr, Kelley RO, Ren J. Dietary iron deficiency induces ventricular dilation, mitochondrial ultrastructural aberrations and cytochrome c release: involvement of nitric oxide synthase and protein tyrosine nitration. *Clin Sci (Lond)* 2005;109:277–286.

49. Juenger J, Schellberg D, Kraemer S, Haunstetter A, Zugck C, Herzog W, Haass M. Health related quality of life in patients with congestive heart failure: comparison with other chronic diseases and relation to functional variables. *Heart* 2002;87:235–241.

50. Enjuanes C, Klip IT, Bruguera J, Cladellas M, Ponikowski P, Banasiak W, van Veldhuisen DJ, van der Meer P, Jankowska EA, Comin-Colet J. Iron

deficiency and health-related quality of life in chronic heart failure: results from a multicenter European study. *Int J Cardiol* 2014;174:268–275.

51. Comin-Colet J, Enjuanes C, Gonzalez G, Torrens A, Cladellas M, MeronoO, Ribas N, Ruiz S, Gomez M, Verdu JM, Bruguera J. Iron deficiency is a key determinant of health-related quality of life in patients with chronic heart failure regardless of anaemia status. E*ur J Heart Fail* 2013;15:1164–1172.
52. Jankowska EA, Rozentryt P, Witkowska A, Nowak J, Hartmann O, Ponikowska B, Borodulin-Nadzieja L, von Haehling S, Doehner W, Banasiak W, Polonski L, Filippatos G, Anker SD, Ponikowski P. Iron deficiency predicts impaired exercise capacity in patients with systolic chronic heart failure. *J Card Fail* 2011;17:899–906.
53. Anker SD, Comin Colet J, Filippatos G, Willenheimer R, Dickstein K, Drexler H, Luscher TF, Bart B, Banasiak W, Niegowska J, Kirwan BA, Mori C, von Eisenhart Rothe B, Pocock SJ, Poole-Wilson PA, Ponikowski P. Ferric carboxymaltose in patients with heart failure and iron deficiency. *N Engl J Med* 2009;361:2436–2448.
54. Ponikowski P, van Veldhuisen DJ, Comin-Colet J, Ertl G, Komajda M, Mareev V, McDonagh T, Parkhomenko A, Tavazzi L, Levesque V, Mori C, Roubert B, Filippatos G, Ruschitzka F, Anker SD. Beneficial effects of long-term intravenous iron therapy with ferric carboxymaltose in patients with symptomatic heart failure and iron deficiency. *Eur Heart J* 2015;36:657–668.

55. González-Costello J, Comín-Colet J. Iron deficiency and anaemia in heart failure: understanding the FAIR-HF trial. *Eur J Heart Fail.* 2010;12(11):1159–1162.

56. Drozd M, Jankowska EA, Banasiak W, Ponikowski P. Iron therapy in patients with heart failure and iron deficiency: review of iron preparations for practitioners. *Am J Cardiovasc Drugs*. 2017 Jun;17(3):183-201.

57. Klip IT, Jankowska EA, Enjuanes C, Voors AA, BanasiakW, Bruguera J, Rozentryt P, Polonski L, van Veldhuisen DJ, Ponikowski P, Comin-Colet J, van der Meer P. The additive burden of iron deficiency in the cardiorenal-anaemia axis: scope of a problem and its consequences. *Eur J Heart Fail* 2014;16:655–662.

Annexures

PROFORMA

Name :

Age/Sex :

OP number :

History:

Symptom	Yes/No	Symptom	Yes/No
Breathlessness		Palpitations	
Orthopnoea		Dizziness	
Paroxysmal nocturnal dyspnea		Syncope	
Reduced exercise tolerance		Nocturnal cough	
Ankle swelling		Wheezing	

NYHA classification :

Hospitalisation, acute coronary syndrome or coronary revascularisation over last 30 days : Yes/No

Any acute/chronic illness other than heart failure that may influence iron metabolism : Yes/No

Blood transfusions, iron supplements, erythropoietin therapy in the last 6 months : Yes/No

Anti-failure pharmacotherapy for >30 days : Yes/No

Aspirin for >30 days : Yes/No

Anticoagulation for >30 days : Yes/No

Active bleeding over last 6 months : Yes/No

Physical examination:

Sign	Yes/No	Sign	Yes/No
Elevated JVP		Cardiac murmur	
S3		Peripheral oedema	
Laterally displaced apical impulse		Ascites	
Hepatojugular reflux		Hepatomegaly	
Tachycardia		Weight gain/loss	
Tachypnoea		Pleural effusions	
Irregular pulse		Pulmonary crepitations	
Oliguria		Cold extremities	

Echo findings:

Ejection fraction :

Specific aetiology for heart failure : Yes/No
(eg. valvular heart disease, congenital heart disease)

Investigations:

Haemoglobin :

Serum ferritin :

Serum iron :

Total iron binding capacity :

Transferrin saturation :

INFORMATION SHEET

We are conducting "**A HOSPITAL-BASED CASE-CONTROL STUDY ON IRON DEFICIENCY AND ANAEMIA IN CHRONIC HEART FAILURE WITH REDUCED EJECTION FRACTION**" among patients attending Rajiv Gandhi Government General Hospital, Chennai. Your co-operation to undergo relevant investigations as per need may be valuable to us.

The purpose of this study is to find the prevalence of iron deficiency in patients with heart failure with preserved ejection function.

We are selecting certain cases and if you are found eligible, we would like to perform extra tests and you will be subjected to a non-invasive procedure like echocardiography which in any way do not affect your final report or management.

The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of Investigator

Signature/left thumb impression of Participant

Date:
Place:

PATIENT CONSENT FORM

Study Detail	:	**"A HOSPITAL-BASED CASE-CONTROL STUDY ON IRON DEFICIENCY AND ANAEMIA IN CHRONIC HEART FAILURE WITH REDUCED EJECTION FRACTION"**
Study Centre	:	Rajiv Gandhi Government General Hospital, Chennai.
Patient's Name	:	
Patient's Age	:	
Identification Number	:	

Patient may check (√) these boxes

a) I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction. ❑

b) I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected. ❑

c) I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study. ❑

d) I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms. ❑

e) I hereby consent to participate in this study. ❑

f) I hereby give permission to undergo detailed clinical examination and blood investigations as required. ❑

Signature/thumb impression

Patient's Name and Address:

Signature of Investigator

Study Investigator's name:
Dr. Tanuj Moses Lamech

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

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CERTIFICATE OF APPROVAL

To
Dr.Tanuj Lamech
Post Graduate in MD General Medicine
Institute of Internal Medicine
Madras Medical College
Chennai 600 003

Dear Dr.Tanuj Lamech

The Institutional Ethics Committee has considered your request and approved your study titled **"A HOSPITAL – BASED CASE – CONTROL STUDY ON IRON DEFICIENCY AND ANAEMIA IN CHRONIC HEART FAILURE WITH REDUCED EJECTION FRACTION " - NO.20012017 (II).**

The following members of Ethics Committee were present in the meeting hold on **19.01.2017** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.M.K.Muralidharan,MS.,M.Ch.,Dean, MMC,Ch-3	:Deputy Chairperson
3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4.Prof.B.Vasanthi,MD., Prof.of Pharmacology.,MMC,Ch-3	: Member
5.Prof.A.Rajendran,MS, Prof. of Surgery,MMC,Ch-3	: Member
6.Prof.N.Gopalakrishnan,MD,Director,Inst.of Nephrology,MMC,Ch	: Member
7.Prof.Baby Vasumathi,MD.,Director, Inst. of O & G	: Member
8.Prof.K.Ramadevi,MD.,Director,Inst.of Bio-Che,MMC,Ch-3	: Member
9.Prof.R.Padmavathy, MD, Director,Inst.of Pathology,MMC,Ch-3	: Member
10.Prof.S.Mayilvahanan,MD,Director, Inst. of Int.Med,MMC, Ch-3	: Member
11.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
12.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
13.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary-Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

URKUND

Urkund Analysis Result

Analysed Document: Thesis - Urkund.docx (D31042786)
Submitted: 10/5/2017 2:58:00 PM
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Sources included in the report:

Portfolio case 2 (2).docx (D16699460)
https://link.springer.com/article/10.1186/1471-230X-14-184

Instances where selected sources appear:

3

PLAGIARISM CERTIFICATE

This is to certify that this dissertation work titled **"A HOSPITAL-BASED CASE-CONTROL STUDY ON IRON DEFICIENCY AND ANAEMIA IN CHRONIC HEART FAILURE WITH REDUCED EJECTION FRACTION"** of the candidate **Dr. TANUJ MOSES LAMECH**, with registration Number **201511009** for the award of **M.D** in the branch of **GENERAL MEDICINE.** I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **1 percentage** of plagiarism in the dissertation.

Guide & Supervisor sign with Seal.

MASTER CHART - CASES

S. No.	Age	Sex	Ferritin	Transferrin saturation	Haemoglobin	NYHA class	Taking aspirin/clopidogrel	Taking acitrom
1	57	Male	115.3	23	13.7	1	Yes	No
2	64	Female	82.5	24	11.8	3	No	No
3	59	Male	81.5	12	11.6	2	Yes	No
4	72	Male	164.7	19	12.5	2	No	No
5	64	Male	395.9	23	13.2	4	No	No
6	66	Female	59.8	8	10.7	4	Yes	No
7	69	Male	73.5	7	11.9	1	Yes	No
8	83	Female	84.6	27	10.4	3	No	No
9	77	Male	67	8	11.7	3	Yes	No
10	42	Male	166.8	16	13.2	2	Yes	No
11	47	Female	352.9	21	12.6	2	No	No
12	57	Male	49.8	19	13.6	1	Yes	No
13	49	Female	173.9	17	10.3	2	No	No
14	64	Male	62.8	19	11.8	2	Yes	No
15	57	Male	53.8	19	13.9	2	Yes	No
16	69	Female	57.2	19	11.9	2	Yes	No

S. No.	Age	Sex	Ferritin	Transferrin saturation	Haemoglobin	NYHA class	Taking aspirin/clopidogrel	Taking acitrom
17	53	Female	139.2	18	10.9	2	No	No
18	66	Male	63.7	17	11.4	2	Yes	No
19	73	Male	68.5	11	12	4	Yes	No
20	66	Female	60.8	18	10.9	3	No	No
21	68	Male	76.1	15	13.7	4	Yes	No
22	72	Female	179.9	18	11.4	4	Yes	No
23	58	Male	96.5	23	13.5	4	No	No
24	70	Female	99.4	25	11.3	3	No	No
25	56	Male	162.7	25	13.3	2	Yes	No
26	52	Male	54.8	18	13.5	3	Yes	No
27	63	Female	75.1	17	10.1	3	Yes	No
28	51	Female	326.7	22	12.7	2	Yes	No
29	60	Male	412.6	35	11.6	1	No	No
30	60	Female	88.8	17	11.9	4	No	No
31	41	Male	184.8	19	13.9	2	Yes	No
32	68	Male	193.9	19	12.5	3	No	No
33	54	Female	37.5	17	10.8	4	Yes	No

S. No.	Age	Sex	Ferritin	Transferrin saturation	Haemoglobin	NYHA class	Taking aspirin/clopidogrel	Taking acitrom
34	68	Male	317.9	33	13.2	1	No	Yes
35	60	Female	126.2	25	12.4	3	Yes	No
36	73	Male	68.8	14	13.6	2	Yes	No
37	64	Female	157.8	16	11.5	1	Yes	No
38	62	Male	472.6	22	13.7	2	Yes	No
39	66	Male	346	37	14.2	2	Yes	No
40	59	Female	67.5	13	10.4	2	Yes	No
41	77	Male	182.7	16	12.1	2	Yes	No
42	62	Female	218.2	18	9.6	2	No	No
43	61	Male	53.8	9	12.6	2	Yes	No
44	72	Female	48.2	6	12	2	Yes	No
45	56	Male	137.8	24	13.8	1	Yes	No
46	58	Female	312.1	35	13.1	2	No	No
47	62	Female	241.9	15	10.6	3	Yes	No
48	50	Male	111.3	23	13	1	Yes	No
49	47	Female	376.2	32	12.3	2	Yes	No
50	55	Male	77.8	14	13.6	2	Yes	No

MASTER CHART – CONTROLS

S. No.	Age	Sex	Ferritin	Transferrin saturation	Haemoglobin
1	57	Male	64.8	18	11.1
2	55	Male	331.1	25	13.9
3	83	Male	242.2	18	13.6
4	53	Male	233.8	22	13.6
5	46	Male	365.5	22	13.2
6	41	Female	132.5	18	11.8
7	46	Female	452.6	25	12.5
8	63	Male	253.2	21	13.2
9	68	Male	395.9	26	13.6
10	49	Female	235.4	19	11.6
11	43	Male	84.9	19	11.8
12	65	Female	85.6	17	8.9
13	66	Male	162.2	24	13.3
14	73	Male	70.7	18	13.1
15	64	Male	185.5	21	13.8
16	71	Male	312.1	23	13.6
17	72	Male	157.3	22	13.3

S. No.	Age	Sex	Ferritin	Transferrin saturation	Haemoglobin
18	68	Male	252.2	17	14.1
19	63	Female	371.4	21	12.6
20	73	Female	326.5	23	13.2
21	70	Male	196.9	23	13.2
22	67	Female	59.7	18	11.5
23	50	Male	226.8	22	13.2
24	56	Female	195.6	17	7.2
25	72	Male	413	25	14.2
26	62	Male	369.1	26	15.1
27	51	Male	71.1	16	13.7
28	57	Female	373.7	25	13.2
29	69	Male	385.5	24	13.7
30	54	Female	319.8	26	12.7
31	74	Male	363	25	14.5
32	53	Male	136.9	23	13.3
33	61	Male	295.9	23	13.8
34	46	Female	264.3	18	9.9
35	73	Male	362.9	24	13.6
36	52	Male	245.2	24	14.6

S. No.	Age	Sex	Ferritin	Transferrin saturation	Haemoglobin
37	64	Male	245.4	22	13.9
38	58	Male	182.4	23	13.7
39	70	Female	184.2	14	11.4
40	68	Female	79.3	16	9.5
41	49	Male	363.1	23	14.1
42	47	Male	332.8	20	14.9
43	56	Male	275.4	17	8.6
44	51	Female	328.9	25	12.1
45	63	Male	233.6	17	12.8
46	69	Female	350.8	26	12.9
47	44	Female	149.7	17	10.6
48	74	Male	121.1	21	13.4
49	56	Male	231	21	13.8
50	61	Female	253.2	15	10.4